AN ASSESSMENT OF THE PREDICTIVE VALUE OF LABORATORY STUDIES IN THE MANAGEMENT OF PERIPHERAL NERVE INJURIES

By

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Haec sic pernosces parva perductus opella;
Namque alid ex alio clarescet, nec tibi caeca
nox iter eripiet quin ultima naturai
Pervideas: ita res accendent lumina rebus.

Lucretius, *De Rerum Natura*, 1:1114–1117

STATEMENT OF ORIGINALITY

I confirm that I was the originator of the idea for the experiments, principal investigator and author of all of the work presented in this thesis.

All of the work presented here was financed by externally refereed research grants awarded *solely* to myself.

Where collaboration has taken place, this has been with Surgical Trainees, Research Students or Honours Students under my supervision or occasionally colleagues acting as anaesthetist or human surgical advisor.

I have not previously submitted this thesis at this or at another University.

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ABSTRACT

THE hypothesis underlying the work to be presented here is: '...that a large animal (sheep) may successfully be used as a model for peripheral nerve injury, repair and regeneration and that laboratory studies using this model may furnish information useful in predicting clinical outcome'.

There are nine chapters. In the first chapter the history of nerve regeneration is briefly discussed. The current conflicts and uncertainties are also outlined along with a summary of the objectives of the present study.

The second chapter is a critique of the use of the sheep as a model for nerve injury and repair and a comparison of this large animal model with small animal models such as the laboratory rat. The conclusion is that the ovine model is a nearer representation of the human case than small animal models.

In the third chapter the various clinical and laboratory tests which may be used to evaluate the outcome of nerve injury and repair are discussed. Their theoretical advantages and disadvantages are discussed in detail and a case made for their use either in clinical practice or in more comprehensive laboratory studies notwithstanding that the methods used in the laboratory may be wholly inapplicable in the clinical setting. The questions posed by their use, and the way in which answers might be sought are outlined. In the following chapters experiments are described each with a view to answering these questions.

Chapter 4 is a consideration of the case of a mixed (median) nerve and in Chapter 5 a purely motor (facial) nerve is investigated using the tests outlined in Chapter 3. In Chapter 6 there is a discourse upon the use of a technique for estimating the

distribution of conduction velocities (CV_{Dist}) in the human. This is an offshoot of the ovine work described in Chapters 4 and 5 and is presented as a prospective clinical test for general use in the assessment of neuropathies and nerve injuries.

In Chapter 7 the special case of obstetrical brachial plexus injury is discussed in the light of the clinical and laboratory tests which may be used to assess it and a similar treatment is given in Chapter 8 to nerve injuries complicated by cavitation, fibrosis, haematoma, long-bone fracture and arterial injury.

In the final chapter (9), all of the above are discussed and the value of both the ovine model and the tests described in Chapter 3 are evaluated.

It is concluded that the ovine model is an excellent substrate for the evaluation of the pathophysiology of nerve injuries, surgical methods for their repair and of the tests which may be used to assess the outcome of these procedures.

Particular thanks are due to Miss Gail Valler who has been a loyal and dedicated technician for many years; to Dr Roger Mallion, my 'mathematical crutch' in times when my limited education in that subject has left me undone, and to Dr Thomas Gilchrist who introduced me to 'technology' and tolerated my appalling golf.

The third group whom I acknowledge contains those whom I am said to have educated: in particular generations of postgraduates reading for MPhil, MS/ChM, PhD and MD degrees. I hope and believe the education process to have been reciprocal.

Today in the rush to mediocrity-through-uniformity, the notion of 'wisdom' has been foresaken in the desire to test 'core knowledge' (whatever that is), usually by the intellectually undemanding process of ticking boxes. Writing sentences and defending onself *viva voce* is of the past and now the future for original research, even as a job discriminator, looks bleak for medical trainees. Hippocrates knew the risks of concentrating upon factual knowledge at the expense of acquiring wisdom when he said: ἰητρος φιλοσοφος ἰσυθεος[1]. Doctors who want to write theses must be doctors who think and the exercise of taking time out to do research is worth preserving for that reason alone.

My final thanks are to the Charitable Bodies who have considered my work worth supporting. They are too numerous to list all here but especial mention must be made of Giltech Ltd. It is sad that universities now underrate such help because charitable grants do not produce the golden calf of 'overheads'.

I am grateful to all of these and hope that I have repaid their trust and generosity.

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[1]In his native Ionic dialect:or ἰατρος φιλοσοφος ἰσοθεος for the Attic purist.

CONTENTS

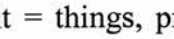
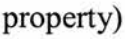
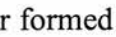
CHAPTER 1 — NERVES & NERVE REPAIR

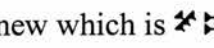
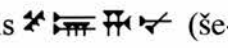
᾽Εν ἀρχῇ ἦν ὁ λόγος

NERVES AND SINEWS.

It is interesting to speculate upon the point in history when the idea of a peripheral nerve and its function emerged. Today, in the culinary and butchery trades, the word 'nerve' is still used collectively for a variety of anatomical structures including tendons, ligaments and bands of condensed connective tissue as well as for nerves themselves. This is an old usage, and it is not apparent at what point in history a distinction was made between nerves as we recognize them today and these other 'sinews'; but it may be later than one might expect.

The Sumerian dynasty was the oldest literate civilization and there is no clear evidence that the Sumerians had any specific word for either a nerve or a sinew although it is clear that the study of Anatomy had its beginnings there.

Although it is often assumed — presumably because of the pains taken over mummification — that Anatomy and Physiology were well developed in the ancient Egyptian civilization, in fact, this was not the case and indeed dissection was prohibited. It seems that the ancient Egyptians viewed the body as a series of distinct parts  (ḫt = things, property) suffused with blood and other fluids  (snf) which together formed an holistic whole  (hꜣw = environment or kindred) or  (ḫʿ = member, piece of flesh). It is noteworthy that they had distinct words for the heart  (ib) but for little else, especially internal organs, other than the limbs, the eye, genitalia and body parts used as food.

The parallel development of Mesopotamian civilization was more concerned with the details of both anatomical and physiological structures and processes. In both the Babylonian and Assyrian Empires the oldest of the Semitic languages, Akkadian, was spoken and was written using cuneiform characters. There is a clearly defined word for a sinew which is  (še-er-ḫa- nu), or possibly  (ši-ir-a-nu) in other dialects. There is no suggestion of this term's ever having been used in the present-day sense of a conducting nerve.

Most of the important hieroglyphic and cuneiform inscriptions that have been studied were created at the times when the Egyptian and Babylo-Assyrian civilizations were flourishing, although hieroglyphic and especially hieratic/demotic inscriptions were still in use in the Ptolomaic period[1]. This is in contrast to the Hebrew and Aramaic scriptures as the Jewish tribes had a well- developed tradition of handing down their stories verbally but a poor tradition of scripture. The oldest Jewish scriptures available today are the Qumran scrolls, though these derive from the Essene civilization (200 B.C.– A.D.100) The traditional Hebrew text of the Jewish *Tanakh*, is termed the Masoretic text. This was sedulously assembled, codified, and supplied with diacritical marks to enable correct pronunciation, between the 6th and 10th centuries A.D. by scholars at Talmudic academies in Babylonia and Palestine; in an effort to reproduce what they supposed to be the original 'text' of the Hebrew Old Testament from gathered manuscripts and whatever oral traditions were available to them. However their intention was not primarily to translate the Scriptures with historical precision, but to transmit to future generations what they perceived to be the authentic Word of God.

[1] *cf* the Rosetta stone

Exhaustive concordances to the Bible such as that of Strong (Strong 2003) are a great help in tracking down words and their usage. The English word 'nerve' appears not at all in any English version of the Old Testament (OT) or the New Testament (NT) and the word 'sinew' occurs only in the OT three times in the singular and five times in the plural. However it is not clear, in most of these cases, whether the structure being referred to is musculo-skeletal or a true nerve. In all but one[2] of these cases the Hebrew word is גִּיד which has the clear meaning of a sinew in the musculo-skeletal sense[3]. This is distinct from the modern Israeli Hebrew word עָצָב (ʿaṣav) used for a nerve quite specifically, and appearing nowhere in the Bible. It is, however, similar in the derivation of its triliteral root to the ancient and the modern Arabic word for a nerve **or** sinew which is عَصَب (ʿaṣab).

It is when we turn to the Greek text of the OT, The Septuagint (LXX), that further problems arise (Hatch & Redpath 1897; Septuagint 1851). In every case the Hebrew word גִּיד is transliterated by the Greek word νεῦρον but in one particular instance (Genesis XXXII:32)[3] the context implies that this νεῦρον was subjected to a 'numbing effect' a property one would expect of a nerve. Indeed the verb used is the Greek, νάρκειν, the root of the modern words 'narcosis', 'narcotize' etc. The point about all this is that the LXX was written in Ptolomaic Alexandria around 300 B.C. and thus pre-dates the Hebrew Masoretic texts. Unfortunately there are no further instances of the usage of νεῦρον in this context so it is impossible to know precisely what was implied.

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[2] the other word is עֲרַק which is identical in meaning.

[3] See Appendix 1

If we go back in time to the true Classical period, the earliest recorded usage of the word νεῦρον is in Homer[4] and the context is clearly that of 'sinews'. The Lexicon (Liddell & Scott 1968) contains a number of other words derived from the root νεῦρ- and denoting such things as bowstrings and the strings of musical instruments. The idea of a tense filament such as a sinew is quite clear. This sense is preserved in Hippocrates (?469–399B.C.) although he makes a clear distinction between sinew (νεῦρον) and a tendon (τενων) — the latter being used in its proper sense as today[5].

By the end of the first century A.D. it is clear that the present-day functions of a nerve were understood though it is likely that the term νεῦρον was still mostly used indiscriminately to mean either a nerve or a sinew. An important point is made in the writings of Rufus of Ephesus (latter half of first century A.D.) who[6] uses the phrase: νεῦρα κινητικά. While it is tempting to think of this as the first description of a motor nerve it may be no more than a description of the mechanical properties of νεῦρα in the sense of sinews or perhaps tendons. What is, however, more certain, is the meaning of the term νεῦρα πρακτικά αἰσθητικά — a 'nerve capable of perception' — used by Galen (A.D. 129–?199) and even more interesting is the fact that Galen tells us that he is quoting Erasistratus who lived from 315 to 240 B.C. It is unclear what Erasistratus actually wrote because there are only a few fragments left today but it is known that he was a scrupulous scientist who was well ahead of his time. Galen also uses the term νεῦρον ἀκουστικόν again quite specifically, to refer to what is today the VIII[th] cranial nerve. By the end of the second century A.D., therefore we must assume that the present day ideas about the function of nerves were being

[4] Iliad 16:316 (in the plural — περί δ' ἔγχεος αἰχμη νεῦρα διεσχίσθη.— 'around the spear-point the sinews were torn apart.'

[5] Hippocrates III:17 Περὶ ἀρθρων.— a remarkably up-to-date account of the management of joint injuries and fractures.

[6] In his work: περὶ ὀνομασιάς (211)

formulated. In the dark ages which were to follow, progress was slow indeed and even halted completely (Ochs 2004).

THE RENAISSANCE AND ENLIGHTENMENT

During this period many discoveries were made which greatly improved the study of the nervous system. Perhaps it was in the field of anatomy that most study was concentrated and the efforts of Versalius and DaVinci must not be forgotten. Physiology moved at a slower pace, but in the context of the present study it is important not to overlook the work of Thomas Willis (1621–1675) in Oxford. In his two major neurological works: '*Cerebri Anatome*', published in 1664 and the delightfully entitled '*De Anima Brutorum*' (On the Soul of Brutes) of 1672, he effectively summrized the state of neurology at the time but more importantly suggested concepts which were to gain recognition at later times.

The age of scientific determinism may be said to have begun truly in 1687 with the publication of the first edition of Newton's '*Principia*'. There was great productivity in both physical and biological sciences. Two local contributions deserve mention: Alexander Munro II, (1733–1817), Professor of Anatomy at Edinburgh University, published his '*Observations on the Structure and Functions of the Nervous System*' in 1783 and Charles Bell (1774–1842) best known for the *Bell–Magendie Law*, his '*A System of Operative Surgery founded in Anatomy*' in 1814. It is noteworthy, however, that the latter of these makes no mention of nerve repair.

A DEFINITON OF A NERVE

The first clear definition of a nerve which was made available to the general public in England is to be found in Dr Johnson's Dictionary of the English Language of 1755, (Johnson 1755).

Nerve – The organs of ſenſation paſſing from the brain to all parts of the body.

It may be helpful as a 'wiring-diagram' but is unhelpful as a generalization, for no mention of motor nerves is made.

Newton[7] (Newton 1726) had appreciated the different functions of motor and sensory nerves but even the greatest of scientists could not suffuciently detach himself from the teachings of the Church to avoid invoking the nebulous concept of the Holy Spirit as a provider of 'ethereal vibrations'. Such a step had to await the elucidation — also by physical scientists — of electricity. Galvani (1737–1798) published his great work '*De viribus electricitatis in motu musculari commentarius*'in Modena in 1791 and this must be regarded as the foundation stone of electrophysiology.

NERVE REPAIR

Ancient physicians and surgeons may have observed the reunion of fractured bones and speculated that nerves (in the sense of sinews, no doubt) might similarly reunite if given the opportunity. There is no textual authority for this assumption though commentators in the Middle Ages mention prescriptions which they ascribe to Galen for treating nerve injuries by 'agglutination'. Roger of Salerno (*floruit* 1170) is the earlist recorded user of such agglutinating agents based upon egg albumin. His student Gilbert Angelicus (*floruit* 1245) has written that transected nerves so treated by his master could be reunited (*conglutinari*) and regenerated (*consolidari*).

The first recorded advice for suturing a nerve is from Guy de Chauliac (c. 1300–1368) though he is not clear about his choice of suture material. Ambroise Paré (1510–1590) famed as a military surgeon, treated Charles IX for a puncture wound which had divided a nerve. His treatment took a different course for he believed that the wound

[7] see quotation at the end of Chapter 9.

should be kept open so that 'filth may pass freely forth' . A variety of (undoubtedly neurotoxic) agents such as turpentine were used to achieve this end. A detailed account of the outcome is, unsurprisingly, not available — surgical audit had not yet arrived.

To Augustus Volney Waller (1816–1870), must be given credit for the revolutionizing of nerve repair by virtue of his account of the eponymous process of nerve degeneration and regeneration. So was born the novel idea that the difficult part of nerve repair is carried out by Nature, the job of the surgeon being that of optimizing the environment in which the regenerative process may take place. Upon this concept is based the entirety of peripheral nerve surgery.

As has been the case in the twentieth century, the greater attractiveness of the central nervous system led to fashions in its study very much at the expense of the peripheral nervous system and for many years the minds of surgeons faced with treating nerve injuries did not rise above amputation. By the end of the nineteenth century however the idea of nerve repair was fully formulated but severely hampered by the lack of refinement and sophistication in surgical equipment and technique. There are many accounts, mostly of single cases of nerve injury and repair from the last quarter of the nineteenth century: none is of seminal importance. The tendency to report such cases piecemeal has, unfortunately persisted to a greater extent than has been good for the discipline and by the end of the twentieth century it was still a widely fragmented field encompassing orthopaedic surgery, plastic surgery, hand surgery, neurosurgery, otorhinolaryngology, maxillo-facial/dental surgery and even occasionally finding its way into the province of the general surgeon. Given the disparate nature of nerve injuries themselves, this thin spreading of their treatment has meant that there has been very little controlled research from the clinical world compared to that from the

laboratory: and all too little communication between these separate disciplines. The natural history of nerve injuries and their recovery, after repair is, as a result, still quite poorly documented and poorly understood.

From time to time there emerges in science a true visionary after whom the subject advances by a major step and upon whose work future generations can more easily build. In vascular surgery in particular, and experimental surgery in general, such was Alexis Carrel (1873–1944; Nobel Laureate in Physiology 1912). His work on the suturing of blood vessels brought about such advances in surgical technique and technology that the scene was set for a parallel advancement in the surgery of peripheral nerves. Curiously, contemporaneous with Carrel there was such a pioneer in the surgery of peripheral nerves but he has received no accolades and remained in obscurity, possibly as a result of the jealousy of his colleagues. This was Basil Kilvington (1877–1947), who, in the space of a single generation, produced both laboratory experimental work and clinical reportage which was not matched for experimental rigour and scientific insight for another fifty years (Glasby & Hems 1993; Kilvington 1905; Kilvington 1907; Kilvington 1908; Kilvington 1909; Kilvington 1912). Kilvington clearly upset the medical 'establishment' in Melbourne by daring to combine clinical practice with the grubby activities of the animal laboratory; and to make things worse, he was almost always correct in his conclusions and in his forcasting. If better technical facilities had been available it seems most likely that Kilvington's work, had it been more widely read, would have transformed peripheral nerve surgery. Even today, his work is rarely quoted though this perhaps is a means of avoiding embarrassment for many researchers who, had they read Kilvington's work, would have cause to doubt the originality of their own.

The subsequent milestones of peripheral nerve surgery in the twentieth century not surprisingly coincided with the two world wars. In the First World War some progress was made but the legacy of powerful and dogmatic voices such as Platt's (Platt 1919; Platt 1921) may have proved to be less than helpful[8]. In the Second World War the large and scientifically determined project led by Sir Herbert Seddon and culminating in the Medical Research Council's Report of 1954 (Medical Research Council 1954) must be considered one of the finest and most informative pieces of planned and coördinated medical research ever: it is the 'Old Testament' of peripheral nerve studies. A New Testament (perhaps even a Messiah) has yet to emerge, though there has been a great deal of research and an even greater amount of anecdotal reportage in the last fifty years. For once, taking its impetus from the MRC Report, Europe has led the way with the original and pioneering work of Millesi, Narakas, Bonney, Birch, and Lundborg to mention the most illustrious[9]. America, despite its wealth, has produced no such luminaries and has been the source of work which is generally of a more derivative nature, though, nevertheless, contibuting significantly, especially in the 'applied' field.

CONFLICTS AND UNCERTAINTIES

While the last fifty years have seen much activity in the field of peripheral nerve research, sadly, it may be concluded that while we now better understand the events which take place when a nerve is injured and repaired, we are not able to offer patients much of an improvement in terms of outcome. Despite scientific advances,

[8] See Chapter 7

[9] At the same time we must not forget the fundamental work of Hodgkin and Huxley, Katz, Sherrington, AV.Hill, E.D.Adrian and colleagues.

the practical benefits of research in the last fifty years have been confined largely to technological advances — these have come from surgeons and those who develop surgical products. Synthetic suture materials and needles, assisted vision and microsurgical techniques have been responsible for the major advances. In addition more accurate diagnosis, the recognition of the need for early repair and better post-operative care have played their part. It seems probable that in this respect a plateau has been reached. We must look elsewhere, most probably to the notion of 'enhancement' of what we already have by whatever physical, chemical or molecular means become available, if we are to improve nerve regeneration further. This moves research outside the field of surgeons and collaboration with scientists in other disciplines will be required. Today, in the United Kingdom the universities are in disarray and there is an acute shortage of doctors. The country, since the election of the current Labour Government in 1997 has moved towards what can only be described as an 'elected dictatorship' and the principal objective of the power-base is regulation of all things at the expense of recognizing individualism. This has rapidly percolated down into surgery where a much truncated training has jetisoned the idea of apprenticeship for 'course teaching' the beloved and 'politically correct' idol of the 'educationalist'. Hence, experience counts for little against the possession of 'qualifications' often of a most unscholarly kind. In practical terms this means that surgeons of the future will learn but one 'correct'[10] way of dealing with any particular problem — a disastrous strategy for dealing with such an heterogeneous problem as nerve injury. Research is to be virtually non-existent in the plans for the surgical training of the future which would be confined to the most rapidly possible means of acquiring the ability to grind patients through the mill of the National Health Service

[10] i.e. not resulting in litigation and as cheap as possible.

at minimum cost. Thus, at a time when the purely surgical aspects of peripheral nerve surgery may be supposed to have reached a plateau and it is the expertise of neuroscientists and molecular biologists which may most usefully be harnessed to improve the lot of nerve-injured patients, the route for collaboration has become a dead-end. These scientists, however great their skills, cannot reasonably be supposed to understand the clinical needs of nerve-injured patients. The surgeons, hamstrung by the 'politically correct', simplistic and monotonic nature of their training will likewise have no idea of what research may offer to the next generation or even to their own. Science, like so much else, follows fashions and these must be exploited to good cause if they are to be worthwhile. This very sadly, seems unlikely in the most deserving field of peripheral nerve repair.

OBJECTIVES OF THE PRESENT STUDY

The present study is the end-point, for one researcher of twenty years' contemplation of the science of nerve regeneration applied in as practical a way as possible, to the surgery of peripheral nerve repair. It is not in any way intended as an agonal event, rather as a base for future studies — if they are ever destined to happen. Coincidental with this writing will be the closure of the only large animal experimental facility which has been concerned with peripheral nerves. Financial considerations and the pusillanimous response of both government and academic bodies to animal terrorism have assured its demise. The ever-accommodating rat will continue as the mainstay of peripheral nerve research despite the protestations, over many years by the present author, that it is a poor — altogether too obliging — model for a process which is anything but obliging to patients. It is therefore important to examine and record what a large animal has to offer and to consider what tests may be useful both in measuring

outcome and, in the laboratory, allowing predictions to be made which will be useful in clinical practice. The hypothesis for the present study is, therefore:

...that a large animal (sheep) may successfully be used as a model for peripheral nerve injury, repair and regeneration and that laboratory studies using this model may furnish information useful in predicting clinical outcome.

The sheep model will be considered first and compared with other animal models, then the tests which may be used in the clinic and the laboratory for testing outcome after nerve repair will be evaluated critically. Thereafter several experimental systems will be described in which the sheep and the tests are used to investigate models of common nerve injuries seen in clinical practice.

CHAPTER 2 — THE OVINE MODEL FOR EXPERIMENTAL NERVE INJURY AND REPAIR

The mountain sheep are sweeter,
But the valley sheep are fatter.
We therefore deemed it meeter
To carry off the latter.

(Thomas Love Peacock, 1785–1866; *The Misfortune of Elphin*)

RATIONALE FOR THE USE OF SHEEP

SUCH information as may be derived from experimental work upon humans would naturally be expected to be of greater value than that derived from lesser creatures; at least in so far as it may be used to predict events in the human. However this is not always so. Restrictions imposed by, for example, the invasiveness of the experimental technique may so limit what information is gleaned form human cases as to dictate that animal models provide superior results. The crucial factor in such a situation is the choice of animal model. After 18 years of experience in the Edinburgh Peripheral Nerve Research Laboratory (EPNRL) the sheep has been found to be an ideal model where extrapolation to the human clinical situation is proposed for two important reasons. First, the sizes of peripheral nerves in the sheep are relatively close to those which are encountered, damaged, in human surgical practice. Secondly, the previous work alluded to above, has revealed that the behaviour of regenerating nerves in sheep is virtually identical to that observed in humans as far as experimental evidence permits (Drew et al. 1995; Glasby et al. 1995) (Gilchrist et al. 1998; Glasby, Fullarton, & Lawson 1997; Glasby, Fullarton, & Lawson 1998).

The problem with small animal models

The laboratory rat has served peripheral nerve research well over many years. Indeed it would by fair to describe it as the 'mainstay' of such work. Its value is unquestioned where the problem in hand does not involve the dimensions of time and length. In each of these cases the obligingness of rat nerves to regenerate results in a very considerable overestimation of the success of the technique under question. Many reports have been published where gaps of 1cm in the rat sciatic nerve have been considered as models of nerve injuries which, in humans, would represent a proportionately much longer gap requiring repair by grafting. Some published papers have made quite absurd claims when the relative dimensions are considered. This a naïve view and as Gattuso (Gattuso et al. 1989; Gattuso, Glasby, & Gschmeissner 1988; Glasby, Gattuso, & Huang 1988) has shown that in rats, regeneration is possible across gaps of up to 7mm without formation of an overt neuroma and in most instances some degree of recordable transmission across the site is possible. A gap of this size, in relation to the length of the sciatic nerve is very considerable and would correspond to a much bigger gap in humans. It is unsafe, therefore, to assume from evidence from models of this sort, that successful regeneration can be expected to predict like behaviour in more advanced species.

The same may probably be said of rabbits though their greater size does allow one to consider longer distances (Hems & Glasby 1992; Hems & Glasby 1993a; Hems & Glasby 1993b). However rabbits kept together in captivity fight and are very prone to cannibalism of their denervated feet. As a result of this they were found to be rather prone to superficial infections. These factors, taken together with the proportionately high cost of rabbits and the expense of their upkeep, led to their abandonment as an experimental animal in EPNRL.

Other large animal models

Many different animal models have been used in the study of peripheral nerve injury and regeneration. These include rats, rabbits, dogs and cats (Boyd 1964; Cragg & Thomas 1964; Erlanger & Schoepfle 1946; Hursh 1939).

Kline *et al* have compared peripheral nerve regeneration across gaps of 2–3cm, after excision of a 1cm length of nerve in the dog, Rhesus monkey, baboon and chimpanzee (Kline, Hayes, & Morse 1964). This procedure resulted in neuroma formation in the baboon and the chimpanzee but in the dog and in some of the monkeys regeneration occurred across the gap with longitudinally orientated axons and restoration of useful conduction. In a parallel study of crush injury in the peroneal and radial nerves, and after nerve transection and suture repair in the peroneal and ulnar nerves, (Kline, Hayes, & Morse 1964), the chimpanzee exhibited significantly slower remyelination of axons in the distal stump than the other animal models after both types of injury and slower recovery of function after the transection injury with suture repair.

In setting up the present studies it was considered imperative to use a large animal model in order to achieve as a realistic a representation of the human clinical situation as might be possible. The above species all presented drawbacks either of a biological nature or of a logistical nature when use in EPNRL was considered. The ideal species for experiments of this sort would, on theoretical grounds, be the chimpanzee; however, cost and the facilities required made this impossible. The problems were much the same for other species of monkey and there is the additional consideration of so-called 'animal-rights' terrorism which is by no means uncommon and widely supported in the United Kingdom today. The effect of this has been to inflate costs because of the need for increased security. In addition, a craven Labour Government,

uninterested in scientific or any other educational achievement, has sought to alleviate the problem by encasing it in bureaucracy and has so discouraged animal experimentation generally. The level of sentimentality afforded to dogs and cats in the United Kingdom is such that, for the reasons above, they have become almost extinct as experimental animals. These considerations led to a trial using Scottish Blackface ewes (Glasby et al. 1990) where nerves were repaired by various means and assessed after a period of recovery. The dimensions of sheep nerves were found to be of much the same order as in humans and, more importantly, the time-course of recovery was also similar. At around the same time, as a possible alternative, a number of studies was set up in our laboratory to investigate the use of the marmoset monkey (Callithrix iacchus) on the grounds that this was a non-human primate but small enough to be manageable (Gattuso et al. 1988; Glasby et al. 1986d; Glasby, Carrick, & Hems 1992). While these studies proved useful, there was no obvious advantage over the sheep in terms of the time-course of recovery or its nature. These monkeys are very small, being only slightly larger than rats and significantly smaller than rabbits; moreover they had an alarming tendency to become obese under laboratory conditions and this made histological examination of fat-laden nerves an unwanted difficulty. It seemed therefore that they contributed no experimental advantage over lesser large species save the dubious notion that they were genetically closer to humans and this did not seem an appropriate reason to continue with their use.

As a result of this early study and with the added advantage that sheep are plentiful and therefore inexpensive in Scotland, it was decided to use this model for all subsequent work at EPNRL. (Drew et al. 1995; Driscoll, Lawson, & Glasby 2002; Gattuso, Davies, Glasby, Gschmeissner, & Huang 1988; Gattuso, Glasby, Gschmeissner, & Norris 1989; Gilmour, Myles, & Glasby 1995; Glasby,

Gschmeissner, Huang, & De Souza 1986d; Glasby et al. 1986b; Glasby et al. 1986c; Glasby, Gilmour, Gschmeissner, Hems, & Myles 1990; Glasby, Clutton, Drew, O'Sullivan, & Whittle 1995; Glasby, Carrick, & Hems 1992; Glasby, Fullarton, & Lawson 1997; Glasby, Fullarton, & Lawson 1998; Lawson & Glasby 1995; Lawson & Glasby 1998) .

Most of the experiments carried out in EPNRL and its predecessors in Cambridge and London, over the last twenty years have involved either the median, ulnar and sciatic, nerves, the brachial plexus or the facial nerve as a model for human injury. Choice of these models has to some extent been governed by Home Office requirements that the animal should suffer minimum disability (Home Office (H.M.S.O.) 1986). Fortunately these models all coincided with common human injuries and did not produce great disability as used in the experimental models (see individual chapters).

Breeds of Sheep

In Scotland sheep are plentiful and relatively inexpensive to obtain and house. Over seventeen years in EPNRL it has been possible to obtain sheep of a number of breeds and to assess them as experimental models (Henderson 1990). For the most part the Scottish Blackface ewe has been our animal of choice (see below). The available sheep fall into two categories: hill breeds and lowland sheep. The hill sheep (Blackface, Greyface, Cheviot, Finn) are invariably more wild but very hardy. They recover well from anaesthesia but would probably be difficult to use for conscious experiments: at EPNRL we have very little experience of this. Hill sheep are reputedly good mothers and live together in flocks without quarrelling. They are usually smaller than the lowland breeds and their nerves are a little smaller than those of humans. The bigger lowland sheep (Suffolk, Dorset, Texel, Booroola, Friesian) are more solitary and more docile. They have bigger nerves but take a longer time to

recover normal behaviour after anaesthesia. In particular they are slow to recommence eating and this can be a major welfare problem. When conscious they are much more placid than the hill breeds. As far as experimental work in the EPNRL is concerned there appear to be relatively few differences between the breeds and none which was found to be significant in respect of the mechanism of nerve regeneration. This proved a salvation during the epidemic of foot-and-mouth disease in 2001 when availability and movement of sheep was severely restricted and recourse had to be made to the use of whichever breeds in whatever numbers were available locally. Needless to say, the outcome of this appalling tragedy has been a considerable increase in the cost of animals. In Scotland, at least, ewes remain relatively inexpensive and indeed cheaper than many smaller experimental animals (notably New Zealand white rabbits) which have been bred commercially for experimental purposes.

The breeds of sheep with which the experiments described below have been concerned are:

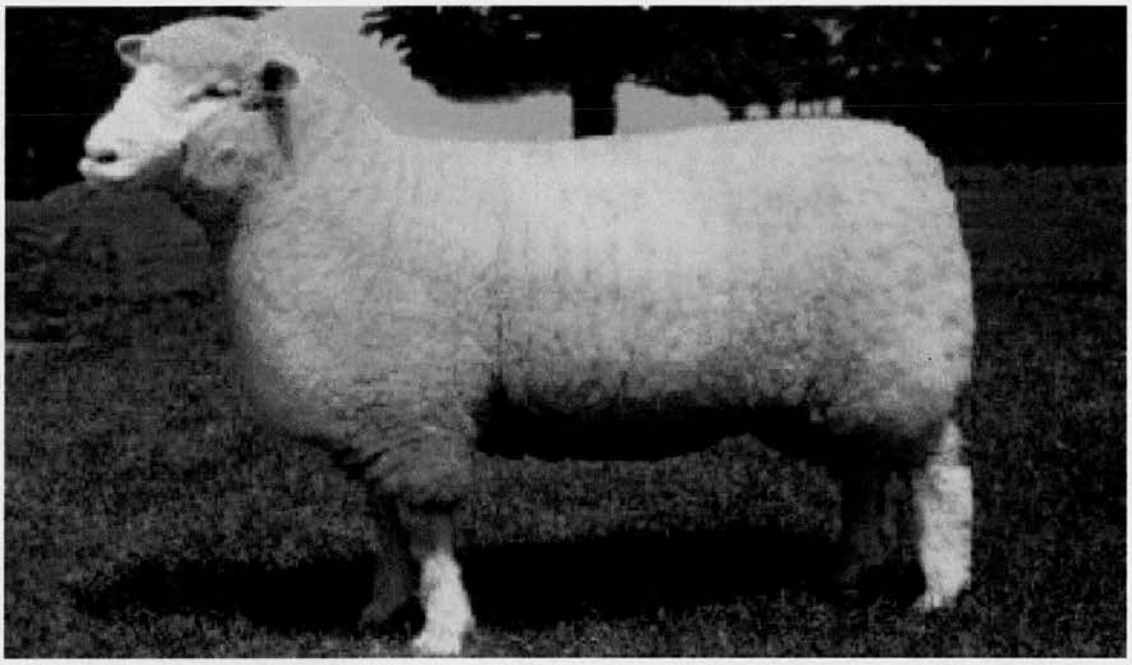
Scottish Blackface

The most numerous of British breeds, the Blackface is found over a wide spectrum of hill and marginal ground throughout Great Britain and Ireland. All Blackfaces are horned, with black, grey or black-and-white face and legs. The Scottish Greyface is a

cross-breed which is essentially similar to the Blackface. The fleece should be free of black fibre, and can vary from short, fine wool to strong coarse wool. Influenced by climate, environment and grazing quality several distinct types have evolved within the breed and are generally identified by the centres at which they are sold. The Perth type, a large-framed sheep with a medium to heavy coat, is found mainly in North East Scotland and Northern Ireland. The Lanark type is dominant in Central and Southern Scotland and the Borders and is that which has been used predominantly in the experiments described in this thesis. It is of medium length, with a shorter, denser coat than that of the Perth type. Over the past 30 years a strong influence of Newton Stewart blood has been introduced; benefiting both milking ability and hardiness. The original Newton Stewart or Galloway type is a compact, burly sheep with a short, thick rain-resistant coat, and is found, in its native South West Scotland, and many of the hard wet areas of the West of Scotland, the Hebrides, and Ireland. Average adult bodyweight: Upland 70kg; Average/good hill 50-65kg; poor hill 45-50kg.

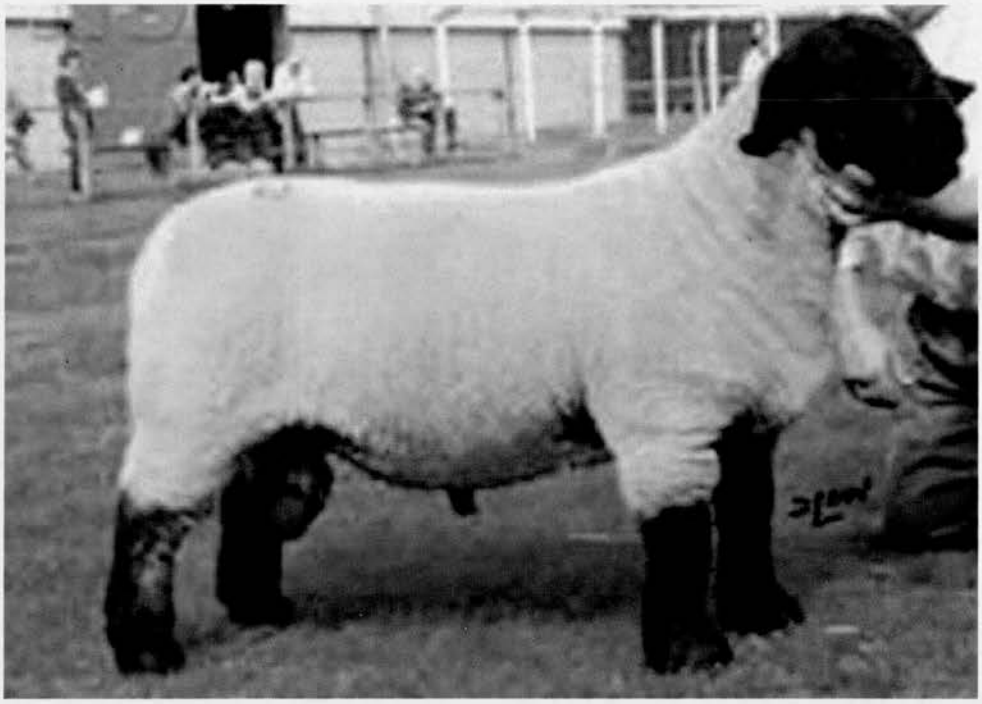
This animal has proved itself ideal for experimental work as it is exceptionally hardy. Recovery from anaesthesia is very rapid and the ewes mobilize very quickly and return to feeding. They are very active at all times but this has not to-date produced any problems with post-operative disruption at the site of repair. Compared with some of the larger breeds, the median and ulnar nerves of the Blackface are admittedly rather small and do not so nearly approximate to human dimensions as in the Dorset, Suffolk and Texel breeds. However, in the Blackface, there is less adipose tissue surrounding the neurovascular bundle and this makes for easier dissection. This is seldom a problem at primary repair but can make dissection of the recovered nerve at assessment quite difficult and time-consuming.

Dorset



Ewes are of medium size and are naturally prolific; skin colour is pink whilst the face, legs and ears are white. The wool is of the highest quality, not only is it fine and densely grown but is particularly white which helps it find a ready market in times of plenty. The average mature ewe weighs 85kg, rams 120kg. These are gentle sheep and easy to handle. Being larger than the Blackface ewes the dimensions of their nerves more closely approximate to those of humans. The Dorset does not tolerate anaesthesia as well, however, and recovery can be drawn-out. Recovery in general is more protracted and although the animals do not suffer overtly they seem less happy than the mountain breeds. These animals have been used for experiments on the facial nerve because they do not have horns. The latter are most usually perfectly situated to obstruct the view through the operating microscope.

Suffolk

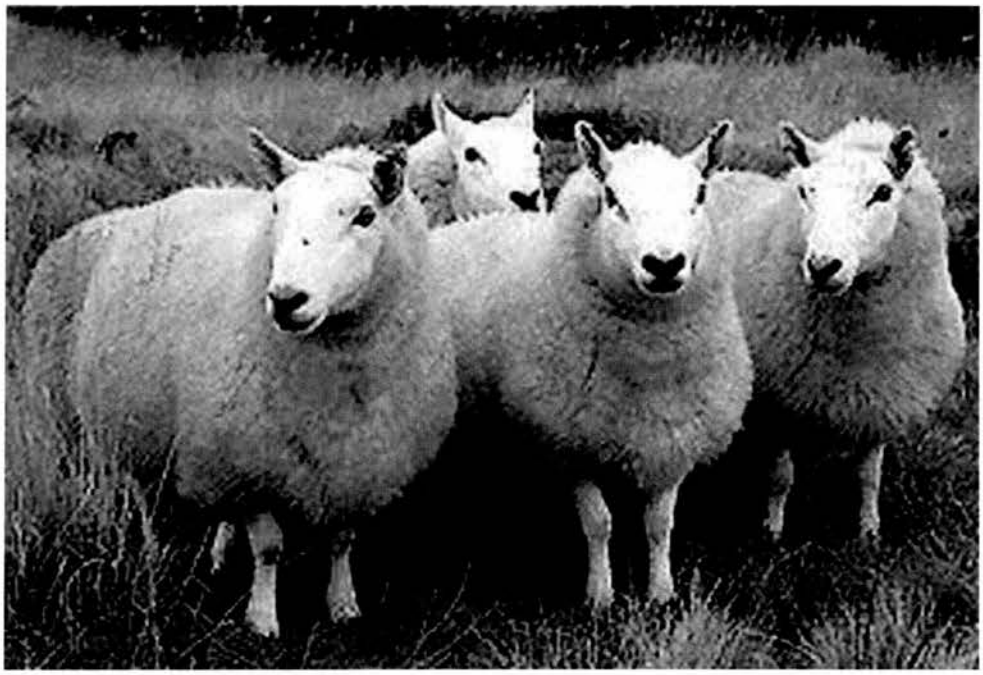


The Suffolk is a polled breed, with a distinctive all-black head and legs, and single colour close-cropped white wool. Independent trials show that Suffolks have the fastest growth-rate of the terminal sire breeds. The average mature ewe weighs 84kg, rams 130kg. The comments regarding recovery from anaesthesia apply here as to the Dorsets but more emphatically. This has not caused trouble during experiments on limb nerves but these ewes did not tolerate operations on the spinal cord or brain well and took a very long time to recover normal behavioural patterns. The principal difficulty was to encourage eating and mobilization after operation. The consequence of starvation in sheep is excessive bloating and splinting of the diaphragm. This may be a reason for terminating the experiment. These are very attractive animals and the largest species; the nerves are all of human dimensions but there can be large amounts of fat surrounding them.

Texel

The head should be covered with fine white hair with only the occasional black spot on the ears. The nose should be black and the ears carried at ten-to-two. A short neck, well fleshed loin, square quarters and well rounded gigots are features of the body. The wool is highly crinkled with a dense, medium length staple and the legs should be of medium bone on deep hard black feet. The average mature ewe weighs 85kg, rams 120kg. Texels are very sturdy and proved to be excellent experimental anumals. They are larger than the Blackface and almost as hardy. They are, however more expensive and less readily available.

Cheviot



Cheviots come from the Cheviot Hills, on the border of England and Scotland. They have white woolless faces and legs, pricked ears, a black muzzle and black feet. They are very alert, active sheep. The Cheviot is a long-wool type, hornless and of reasonable frame. The ewes are born mothers so have few problems with their lambs. They exhibit less foot-rot than other breeds of sheep, though this is of little relevance in experimental conditions. The experience with Cheviots is rather limited but they appear to be another excellent, hardy breed and entirely suitable for experimental use.

Booroola

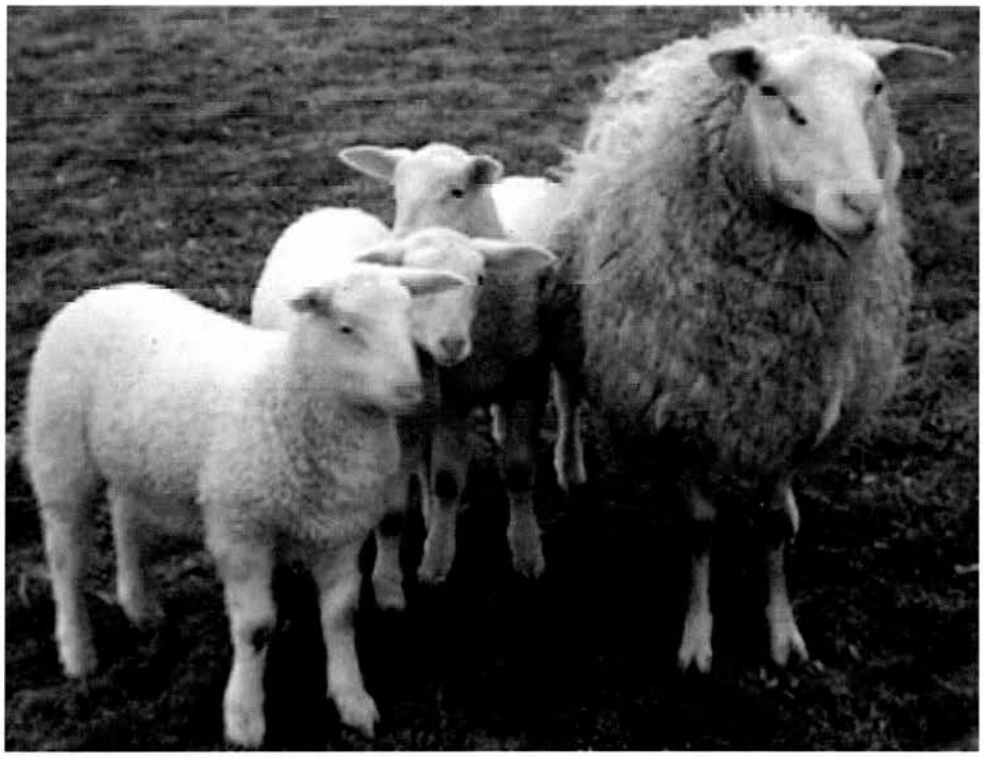
The Booroola Merino was originally developed on the Southern Tablelands of New South Wales. Booroolas differ from the normal Merino in two important ways. First, their fertility is as high as any breed in the world. The number of lambs born per ewe-lambing averages 2.4 with a range from one to six. In crosses with other Merinos, this difference is naturally reduced but half-Booroola ewes on average wean about 20% more lambs than comparable Merinos under the same conditions. Secondly, they have the ability to breed at most times of the year, thus extending the breeding season.

This is a relatively rare breed of sheep but a large flock exists for use in endocrinological experiments by the M.R.C. at the Marshal Building at Roslin where EPNRL is based and where most of the experiments were carried out. During the foot-and-mouth epidemic when sheep could not be bought-in, it was possible to obtain some Booroolas. These are large, hardy sheep which proved to be excellent experimental animals.

Finn

Finnsheep or Finnish Landrace, are native of Finland, The breed is considered to be several hundred years old, descending from the mouflon that live in the wild on Sardinia and Corsica. They are also said to be related to other Scandinavian short-tailed sheep. Their origin is probably related to their high adaptability to the rugged climate and the high-roughage feed available. These are another rarer breed obtained locally as with the Booroolas. They are of a similar size and hardiness to the Scottish Blackface. They do, however, have rather spindly legs which have been found to be slow to heal after surgery and more liable to form contractures during the period of denervation which occurs before reinnervation takes place.

East Friesian



The origin of the Friesian sheep breeds is the region of Friesland extending along the North Sea coast westward from the Weser River in Germany along the north coast of the Netherlands and south to the Schelde river at the border of the Netherlands and Belgium. Offshore is a fringe of islands including the West 'Frisian' Islands belonging to the Netherlands, the 'East Frisian' Islands belonging to Germany, and, to the north, the North 'Frisian' Islands divided between Germany and Denmark.

The family of Friesian sheep breeds are of the marsh-type including the East Friesian Milk Sheep (Deutsches Friesisches Milchschaf) from East Friesland, Germany, and, from the Netherlands, the Dutch Friesian Milk Sheep (Fries Melkschaap) from West Friesland, and to the south, the Zeeland Milk Sheep (Zeeuwes Melkschaap) from Zeeland island. These breeds are similar in appearance, polled in both sexes, with white wool and white faces, ears, and legs all clean of wool. Their most distinctive physical feature is a 'rat-tail', thin and free of wool. Litter size in the East Friesian is

reported as averaging 2.25 lambs with milk yield of 500-700 kg per lactation containing 6-7% milk fat, the highest average dairy milk-yield recorded for any breed of sheep. Wool production is about 4.5 kg per ewe with a clean wool yield of 65% and great fineness. The mature weight of this breed is between 70-90 kg.

The East Friesian is considered to be the world's most productive dairy sheep. They are highly specialized animals which do poorly under extensive and large flock husbandry conditions. These animals fared well in the relatively minor limb operations. Only a few were used in emergency conditions dictated by the foot-and-mouth epidemic. There were no problems but these would not be an obvious choice as an experimental animal where other species were available.

Overall choice of breed

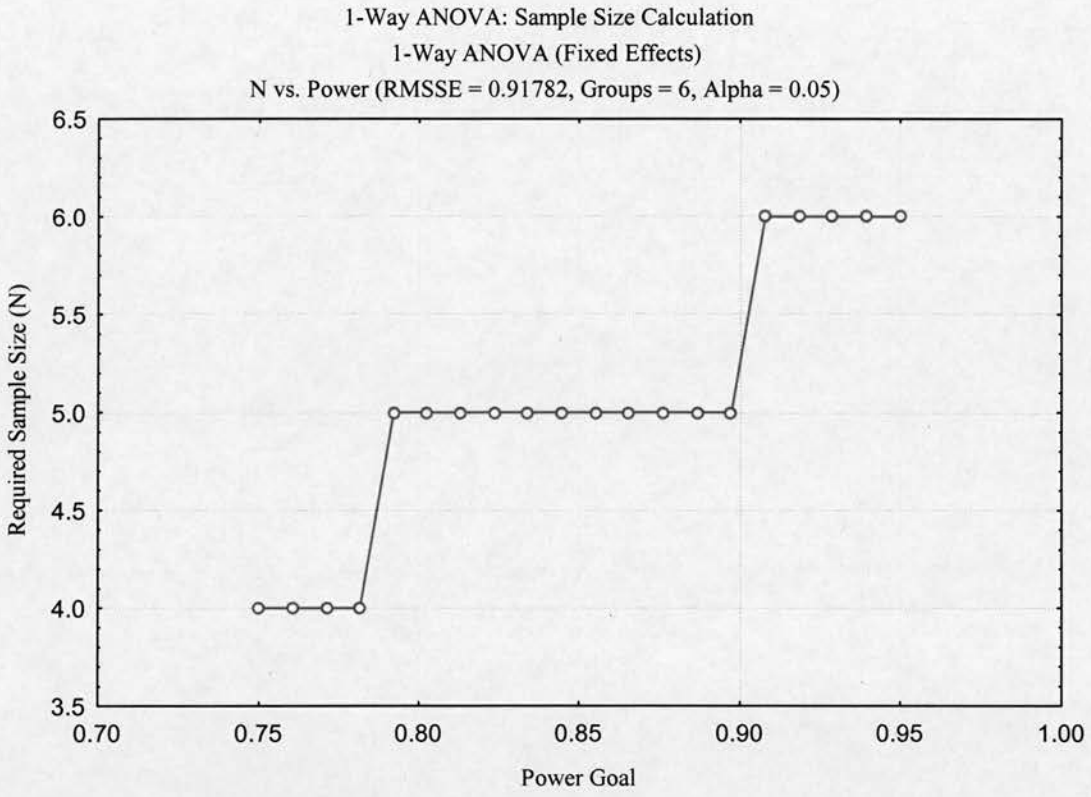
None of the breeds was associated with unsurmountable problems. Three breeds, Scottish Blackface, Texel and Booroola, showed themselves to be excellent experimental animals. A limited experience with Cheviots and Finns suggested they too would be useful. Dorsets, Suffolks and Friesians though acceptable were more likely to present difficulties especially with eating in the post-operative period.

In an ideal situation the Texel might be thought the best model for nerve repair being hardy and of a large size. The rarer Booroola may be equally good. On balance the hardiness, cheapness and local availability of the Scottish Blackface outweighed its small size to make it the animal of choice.

GROUP SIZE

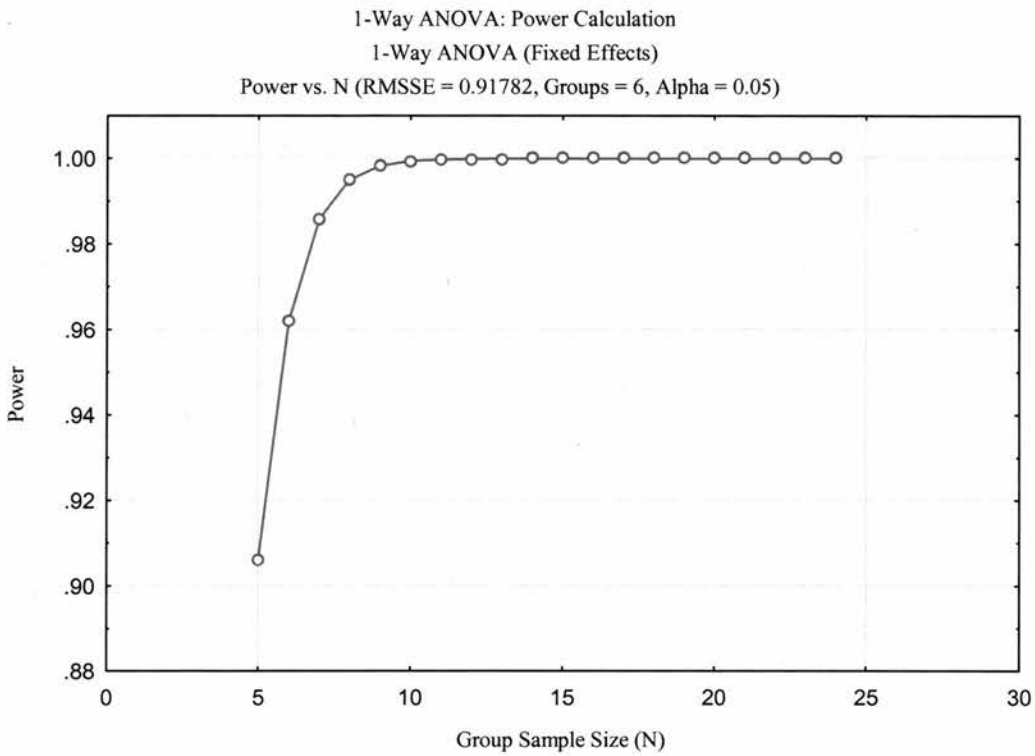
The Act of Parliament governing the experimental use of animals states that a minimum number of animals consistent with a statistically significant result shall be used (Home Office (H.M.S.O.) 1986). A disadvantage of using a large animal model

such as the sheep is that these animals are relatively expensive to buy and maintain especially outside Scotland. However the experience gained at EPNRL over seventeen years has made available a corpus of data from which, by the use of 'power studies', an estimation of the appropriate sample size may be made (Fullarton 1994) The 'Statistica' software (see below) contains a module into which previous results and their standard deviations may be entered in order to produce a graph of sample size against power. Statistical power is defined as: '*The probability of rejecting a false statistical null hypothesis*'. The desirable power to be achieved was 0.9 or better. For example, in a previous experiment maximum conduction velocity CV_{max} in the nerve was measured in groups of 6 animals. In each case the model used in the experiment and the mean value for CV_{max} obtained were: *normal control* 82.55 m s^{-1}, *neurapraxia* 77.80 m s^{-1}, *axonotmesis* 56.25 m s^{-1}, *neurotmesis+epineurial-repair* 40.40 m s^{-1}, *neurotmesis+entubulation-repair* 42.51 m s^{-1}, *neurotmesis+autograft-repair* 35.44 m s^{-1}. The population standard deviation was 21.91 m s^{-1}. With a probability limit of $p<0.05$ ($\alpha = 0.05$) and a 'power goal' of 0.9, the following relationship was obtained:



The graph indicates that the desired power (0.90) for this particular variable could be just (but only just) obtained with a group of five animals. Increasing the group size by one animal would only raise the power by 0.05. However, the problem with a group size of five or even six would be that there would be no latitude for unexpected diminution by death, illness or experimental failure. One must, therefore extend the calculation hypothetically to embrace a sample size which is realistic in that it will ultimately deliver an acceptable statistical power, will 'protect' against unplanned problems and will still fulfil Home Office requirements.

To see the effect of increasing the sample size the same data were plotted with group size on the x-axis and power on the y-axis and in this case entering the number 12 for the group size. This gave the following relationship:



It can be seen that a dramatic change in power takes place between sample sizes of five and ten. Ten animals would give a power of 1.0 with little loss of power if one or two beasts had to be removed from the series — a group of twelve would be a very secure experimental number.

USING THE SHEEP AS AN EXPERIMENTAL ANIMAL

Animal Housing

The experiments described in this work were performed at the Marshall Building at Roslin Institute for Bioresearch, near Roslin, Midlothian: this is a Home Office approved site being a facility shared between the Medical Research Council and the University of Edinburgh and devoted entirely to experimental work with sheep. It had the advantage that the animals could be housed either in Dutch barns or in the surrounding fields. As a consequence of this it was possible to keep large numbers of animals together and they did not have to undergo transportation or a 'settling-in'

period to become accustomed to unfamiliar surroundings. The animals were kept indoors for 24 hours before surgery and were fasted overnight. For routine daily care the animals were looked after by experienced personnel, one of whom also acted as the anaesthetist for the surgical procedures. The technical staff were competent to give drugs by the subcutaneous and intramuscular routes and weight and daily progress charts were kept on all of the animals.

Anaesthesia

Sheep show several important differences from humans in respect of their response to anaesthesia (Clutton & Glasby 1998a; Clutton & Glasby 1998b). In particular they are at risk when lying on their backs from the accumulation of gas in the system of stomachs and this may cause severe splinting of the diaphragm with an increase in intrathoracic pressure and inadequate cardiac filling. This can be rapidly fatal if the rumen is not decompressed with a trochar[11]. As a consequence, it is important to minimize the time which the animal spends in the supine position. Also, anaesthesia in sheep precipitates excessive salivation which is not blocked by atropine and so continuous gentle suction is desirable to remove the saliva. As a consequence of this, adequate fluid replacement must be given intravenously lest the animal become dehydrated. Sheep are unusual also in their response to neuromuscular blockade, (Clutton & Glasby 1998a; Clutton & Glasby 1998b) but this can be overcome by careful titration of the dose. In most of the experiments described here neuromuscular blockade was not needed (and was contraindicated in all of the 'assessment'

[11] There is a graphic description of this condition and its remedy in '*Far from the Madding Crowd*' by Thomas Hardy.

experiments) though it was necessary to use muscle relaxants in operations on the spinal cord and brain in order to remove the possibility of twitching.

The animals were anaesthetized in an anaesthetic room adjacent to the operating theatre. The animal was gently restrained and an area of skin overlying one of the internal jugular veins was shaved using clippers. General anaesthesia was induced with an intravenous injection of thiopentone (0.2ml kg^{-1}) into the jugular vein. The animal was then placed supine on a trolley and its trachea intubated with a cuffed silastic endotracheal tube, under direct vision and with the aid of a long Mackintosh laryngoscope. Correct positioning of the tube was confirmed by auscultation.

The sheep was then transferred to the operating theatre and placed on the operating table in a supine position for access to the median nerve or in the (usually right) lateral position for access to the facial nerve or brachial plexus. The endotracheal tube was connected to a pressure-cycled ventilator (BOC-Manley Promovent) and general anaesthesia was maintained with 0.5 – 2% vaporized halothane, in a carrier mixture of 50% nitrous oxide to 50% oxygen at a flow rate of 6 – 8l min^{-1}. The animal was monitored throughout the procedure using pulse oximetry from the tongue, three-limb-lead ECG and a temperature probe placed in the oesophagus. Depth of anaesthesia was determined by loss of the corneal reflex and observation of the pulse rate (ECG), looking for the development of a tachycardia. Where the brain or spinal cord were the subject of operation or the operation was likely to be prolonged, and arterial pressure line was placed in an auricular artery and a central venous pressure line in the internal jugular vein. Intravenous fluids (0.9% saline, Hartmann's solution or Ringer lactate) were given, by way of a peripheral vein, continuously in response to the CVP or *pro re nata* where no CVP line was present in response to estimated losses from saliva etc.

At the end of the procedure the delivery of halothane and nitrous oxide was stopped and the delivery of oxygen increased. The animal was extubated once it was breathing spontaneously and coughing on the endotracheal tube. For those animals undergoing a non-recovery procedure general anaesthesia was not reversed and a lethal injection of sodium pentobarbitone (140mg kg^{-1}) was administered.

Surgical approaches

Most of the experiments described here involved the median nerve and the surgical approach will be described here in detail along with the procedures involved in creating nerve injuries and in their repair. The additional, special surgical procedures required for the facial nerve are described in Chapter 5 and those for the brachial plexus in Chapter 7. Any additional surgical procedures proper to the tests used in the assessment of experimental outcome are described in Chapter 3 or in the chapters dealing with specific injuries.

Surgical Approach to the Median Nerve

The animal was placed in a supine position with supports on both sides. In order to operate upon the right median nerve in the upper arm i.e. where it lies parallel to the brachial artery, the right axilla reaching onto the chest wall and the right foreleg were shaved using electric clippers. A triangular sandbag was placed under the right side of the thorax and the operating table was rotated to the left. The ewe's right foreleg was abducted and extended and secured so as to create the most obtuse angle between the chest wall and the medial surface of the limb.

All surgical procedures were carried out in a manner exactly to mimic human surgical operations. Sterile operative conditions were scrupulously maintained. In veterinary practice attention to procedural detail is much more lax. In certain cases this is

understandable but it is important to emphasize here that these experiments were designed to test techniques destined for human use and so had to be carried out with that in mind at all times. The skin was prepared with povidone-iodine solution (Betadine, Seton Health Care, England) and the surrounding area covered with sterile surgical drapes. These were secured with towel clips. Bipolar diathermy was used to achieve haemostasis. The abduction and extension of the right foreleg caused visible taughtening of the lower border of the pectoralis major muscle and the ridge so formed was used as a guide for the position of the skin incision. The skin was incised transversely (i.e. cranio-caudally) at right angles to the lower border of pectoralis major. In non-recovery experiments the incision was made at right angles to that described above and extended down the leg. This gave better exposure and avoided scar-tissue from the previous operation. The transverse incision gave poorer exposure but healed very well. It was entirely possible to carry out all microsuturing and entubulation procedures through a 5 – 8cm incision of this kind.

The lower border of pectoralis major was retracted cranially to reveal loose connective tissue which could be easily dissected with fingers to expose the neurovascular bundle of the median nerve, the brachial artery and the ulnar nerve (lying in that order in a cranial → caudal direction). The neurovascular bundle lay on a flat bed of muscle and was covered with thick translucent connective tissue which could easily be divided with blunt-ended fine Metzenbaum scissors. Careful use of the scissors and a nerve hook allowed the separation of the median and ulnar nerves from their beds on either side of the brachial artery. Once the median nerve was adequately exposed, the appropriate injury and repair procedure was carried out as described below.

At the end of the repair procedure after ensuring haemostasis the wound was closed. Interrupted 2/0 polyglactin (Vicryl, Ethilon, UK) sutures were used loosely to approximate the lower border of pectoralis major to the lower border of the axilla (latissimus dorsi, teres major etc.). The subcutaneous tissues were closed with continuous 3/0 polyglactin (Vicryl, Ethilon, UK) and the skin was closed with a continuous 4/0 subcuticular suture of the same material. An antiseptic spray dressing was applied to the wound (Opsite, Smith and Nephew, England). No other dressings were used.

General approach for assessment

In accordance with the terms of the Licence the second operation was a terminal procedure (Home Office (H.M.S.O.) 1986). Therefore, clean but not sterile instruments and drapes were used. As the procedure lasted several hours, the temperature of the animal was monitored throughout the procedure and if necessary warming was carried out with a thermostatically controlled blanket. In all cases intravenous saline was administered continuously as described above in response to measurement of the CVP. Arterial blood pressure was, similarly, measured as described above.

The animal was positioned as described for the first median nerve procedure but the incision was placed parallel to the lower border of pectoralis major and extended into the arm down to a point just above the elbow. A ground electrode was positioned on a shaved area of lateral wall the chest and sutured into place. Efficacy of conduction was improved by use of a small amount of electrode paste. Surface recording electrodes were placed over the flexor carpi radialis (FCR) muscle which is solely supplied by the median nerve. The muscle was easily located and palpated, lying adjacent to the bigger flexor carpi ulnaris (FCU). The cathode was positioned over the

motor point of the muscle (located as that point giving the most perpendicular 'take-off' of the negative phase of the action potential from the isoelectric line) and the reference electrode was placed over the electrically inactive distal tendon.

Dissection of the median nerve was more difficult than in the first procedure as a consequence of scar tissue and a hand-held nerve stimulator proved useful in those cases where location was difficult. The precise site of nerve repair was avoided and the nerve dissected proximally and distally so as to facilitate the application of electrodes and not to risk disruption of the repair site which was encased in reactive tissue. Two bipolar electrodes (proximal anode and distal cathode), were placed under the dissected nerve at the most proximally and distally available sites to serve as stimulating electrodes. The distance between the two cathodes was measured carefully and recorded. The adjacent ulnar nerve was also identified and a 5cm length was excised. This was to prevent unwanted antidromic impulses arising in the median nerve from activating anterior horn cells to produce orthodromic excitation of ulnar nerve territory in the manner of an F wave. Further details of the testing procedures may be found in Chapter 3.

Post-operative Care

An intramuscular injection of 50mg flunixin (Schering Plough, Animal Health), a non-steroidal anti-inflammatory agent, was administered at the end of the procedure for postoperative analgesia. All animals received a single intramuscular injection of 750mg of cefuroxime (Zinacef, Glaxo, UK) on induction of anaesthesia. In operations involving the brain or spinal cord or where extensive use of prosthetic materials occurred or where the dissection had been particularly extensive (brachial plexus), 1g of benzyl penicillin was given intravenously at the start and at the end of surgery and the cefuroxime was continued for five days.

When the animals were conscious they were transferred to a recovery pen. They were positioned upright against a wall of the pen by placing a bale of straw along the opposite flank and a smaller bale of straw to support the head. The animals were closely monitored in the immediate postoperative period until they were mobile and eating. They were kept in the recovery pen overnight and transferred the following day to the barn or outside to a field.

MODELS OF NERVE INJURY IN THE SHEEP

Many models of nerve injuries and their repair have been considered in laboratory investigations stretching back over many years. The basis for all of these has been Seddon's classification of nerve injuries into neurapraxia, axonotmesis and neurotmesis[12] (Birch, Bonney, & Wynn-Parry 1998; Seddon 1943; Seddon 1975; Seddon 1954; Sunderland 1978; Sunderland 1991). There are many criticisms to be made of this classification on theoretical and practical grounds and it is quite clearly inadequate in many situations and misleading in a few. Whatever one's view, however, there is no doubt at all that this is a most useful starting-point for discussion of the subject, simple diagnosis and therefore the design of experiments to investigate methods of repair. Indeed it is still the most widely taught classification for trainee surgeons throughout the world. The object of the experiments described in this thesis has been to create a paradigm of laboratory-based investigations from which it may be possible to make predictions of outcome to be of use to the clinician. There seemed to be no better starting-point than this very basic classification of nerve injuries.

[12] Seddon gave credit for the naming of his three types of injury to Lord Cohen of Birkenhead who was obviously something of a classical scholar.

Neurapraxia

Neurapraxia[13] is characterized by localized demyelination of the nerve and conduction block. There is no subsequent Wallerian degeneration distal to the site of injury as in the more severe insults. In neurapraxia motor nerve conduction studies reveal a decrease in amplitude of the target muscle compound muscle action potential (CMAP) and an increase in latency (Kimura 2001). Neurapraxia is thought to be caused by ischaemia and/or as a direct effect of compression (Driscoll, Lawson, & Glasby 2002). Satisfactory experimental models for neurapraxia had not been described until that of Starritt (Starritt 2005) in which a model of injury was developed using a ligature, composed of biodegradable glass (Giltech, Ayr, Scotland), which was tied around a nerve so as to compress it locally. The composition of the ligature was such that it lost tensile strength over a short time resulting in a transient insult to the nerve. It was shown experimentally that this caused local demyelination without distal Wallerian degeneration and that this corresponded with a an increase in latency from 2.23±0.12 to 3.04±0.11m s^{-1} and a fall in amplitude from 3.87±0.82 to 0.41±0.17 mV with $p < 0.001$, (Starritt 2004).

Axonotmesis

Axonotmesis involves axonal disruption and Wallerian degeneration distal to the site of injury. The endoneurial tubes remain intact, therefore axons should regenerate along their original path. Gutmann et al described a method for producing an experimental axonotmesis in the tibial and peroneal nerves in the rabbit (Gutmann et

[13] Frequently mis-spelt neur***o***praxia [*sic*] in the author's experience even by professors of surgery who edit standard texts and should know better! The splendid anathema fulminating against 'declining standards of literacy' and 'loose thought' by Birch and Bonney (Birch, Bonney, & Wynn-Parry 1998) makes the point vividly and is undoubtedly true.

al. 1942) which used 'fine, sharp, smoothed-faced watchmakers' forceps' to crush the nerve several times at the same spot in order to create a 'transparent thread over 1mm to 2mm'. They confirmed that all fibres had been disrupted by the fact that the distance from the site of injury at which reflexes could be elicited increased with time, in a similar manner to that after neurotmesis and suture repair. They postulated that a smooth-faced forceps was better than a toothed forceps as the latter was more likely to disrupt the endoneurial tubes, and result in a Sunderland Type III or Type IV injury (Sunderland 1978).. A similar method was used by Sanders and Whitteridge (Sanders & Whitteridge 1946) but the time period of application of the crushing force was thought to be of significance. These workers crushed the peroneal nerves of rabbits for ten seconds using smooth watchmakers' forceps.

In the EPNRL a similar method of producing axonotmesis was used with the added refinement of trying to ensure that the compressing force was roughly constant in all cases. In order to achieve this a ratcheted, non-toothed micro-needleholder (Codman, Randolph, Massachussettes, USA) was used. This had fine points of the same dimensions as watchmakers' forceps. It was closed to the second point of the ratchet and maintained for ten seconds (Fullarton 1994).

Differentiation of neurapraxia and axonotmesis in the experimental models

In axonotmesis, conduction in the distal nerve segment will initially be normal because the axons remain excitable. Robinson has suggested that three days after axonotmesis axons and myelin begin to fragment (Robinson 2000), and this is associated with a fall in amplitude of the CMAP which is lost by the ninth day. However degeneration may not be histologically evident for up to fourteen days in humans and in sheep and at this time nerve conduction studies cannot distinguish between neurapraxia and axonotmesis. This is a major problem in clinical practice

(Smith 1996; Smith 1998) and illustrates the importance of timing of investigations. Nerve conduction tests performed too early may underestimate the severity of the injury, as the distal segments of nerves with axonotmesis (and transection) injuries are still excitable. After two weeks however, nerve conduction studies can differentiate between neurapraxia and axonotmesis (and transection) injuries as the distal segments will have undergone Wallerian degeneration. At this time it is possible to differentiate the two types of injury-in-continuity because in neurapraxia, stimulating distal to the lesion will result in a delayed and reduced CMAP whereas in axonotmesis, such stimulation would not elicit a response.

Neurotmesis + repair by epineurial suture

The nerve was transected using a Meyer neurotome. This produces a very clean severance with parallel faces to the nerve stumps. It may be necessary to remove epineurium by a process akin to Rabbinical circumcision in order to prevent its inclusion at the site of coaptation[14]. A rectangle of contrast-material was placed under the nerve and an operating microscope (Wild, Heerbrugg M690, Switzerland), with a sterile cover, was positioned over the nerve. The nerve was viewed through the microscope and checked for the presence of bleeding intraneural vessels. If present these were coagulated with fine bipolar diathermy. Visible anatomical structures such as fascicles and surface vessels were aligned in an attempt to restore the nerve to its original configuration. The nerve was coapted using interrupted 10/0 polyamide epineurial (Ethilon, Ethicon, UK) sutures, placed using microsurgical instruments.

[14] One should never use the term 'anastomosis' in nerve repair because a nerve does not possess a 'stoma'. This term, properly applicable to gut and blood vessels is widely misused by surgeons and this usage is to be regretted.

The sutures were placed in the epineurium at high magnification taking care to avoid the fascicles and tied at a lower magnification.

Neurotmesis + repair by entubulation

Entubulation has both practical and theoretical advantages over suturing techniques. There is no need for microsurgical techniques which require expensive training, consumables and equipment. There is less surgical trauma to nerve ends, and the maintenance of a small gap between the stumps has been postulated as a reservoir for growth factors which may facilitate better regeneration (Kelleher et al. 2001; Lundborg et al. 1982c; Lundborg et al. 1982a; Lundborg et al. 1982b; Lundborg 1993; Lundborg et al. 1997; Lundborg 2000b; Lundborg, Dahlin, & Danielsen 1991; Lundborg & Hansson 1980; Lundborg & Hansson 1981). Possible disadvantages include the retraction of the nerve stumps from the conduit, inflammation and scarring owing to a foreign-body reaction and the need for a second procedure to rectify this (Lundborg 2000a; Lundborg, Dahlin, Danielsen, Hansson, Johannesson, Longo, & Varon 1982a; Lundborg, Dahlin, Danielsen, Longo, Powell, & Varon 1982b; Lundborg, Rosen, Dahlin, Danielsen, & Holmberg 1997; Lundborg & Hansson 1981). In the experiments described here, a flexible wrap was used made from 'controlled release glass' (CRG), fibres matted together with a skeleton of polycaprolactone fibres (Giltech, Ayr, Scotland). CRG is a biodegradable polymer of phosphates of sodium and calcium. The glass dissolves to form salts of its constituent ions and its rate of dissolution may be controlled by altering proportions of these constituent ions (Burnie J. et al. 1981; Burnie J. & Gilchrist 1983; Duff S.R.I. et al. 1984; Gilchrist et al. 1992). Controlled release glass has been demonstrated to be non-toxic and is already in clinical use for example as an antimicrobial dressing for burns.

Previous work using rigid CRG tubes to repair peripheral nerves yielded results comparable with standard epineurial sutured repair (Gilchrist, Glasby, Healy, Kelly, Lenihan, McDowall, Miller, & Myles 1998; Kelleher 2004; Lenihan et al. 1998) . However, these rigid tubes had to be manufactured in predetermined sizes: this made them difficult to place and they were somewhat bulky and fragile. The use of soft wraps which may be cut to size and held in place with either 6/0 sutures and/or tissue glue offers a great advantage in technique, speed and cost.

1. The nerve was transected as before and the two ends placed upon the sterile wrap whose initial size was 4cm by 5cm and which was trimmed to approximately 3cm by 4cm. The two stumps were aligned and glued to the wrap by means of a single drop of glue placed under each stump. Great care was taken that no glue should be present between the cut ends of the nerve. The CRG wrap was then folded over the nerve and secured with 'Tisseel' fibrin glue (Tisseel, Immuno AG, Vienna, Austria). A few further drops of glue were placed on the edges of the wrap where it met the nerve to anchor it securely. Neither microsurgical instruments nor an operating microscope were necessary at any point throughout this procedure.

Neurotmesis + repair by nerve autograft

A number of different techniques have been employed experimentally in the study of nerve repair by means of nerve autografts. In the most general situation where the experiment has been concerned with mechanisms rather than clinical reproducibility, a full-thickness graft has been used. This has usually been made by simply replacing the excised portion of nerve though some degree of rotation may have been allowed to occur in order to disrupt the alignment. This type of graft, of course, would never be encountered in clinical practice and may be expected to give an over-optimistic

measure of outcome. In the clinical repair of large nerves two techniques may be used to overcome the disparity in size between injured nerve and available autograft. Cable grafts (Seddon 1947; Seddon 1975; Seddon & Holmes 1944; Seddon 1954) are created by using several strands of donor nerve in parallel to bridge the gap. Nowadays these are glued together as a bundle with fibrin glue which makes their insertion easier. However no alignment of fascicles is possible.

In order to improve outcome by aligning individual fascicles it is necessary to repair each of these, or at least the major ones, separately. This technique of interfascicular repair was popularized by Millesi (Millesi 1980; Millesi 1981; Millesi 1991; Millesi, Meissl, & Berger 1972) and while it is undoubtedly to be preferred on theoretical grounds, it must be remembered that if performed with poor technique it is likely to result in a worse outcome than the simpler business of inserting a cable graft. In the sheep median nerve there are usually between three and five fascicles which can be repaired in this way by a microsurgeon who is competent to use 11/0 suture material.

Neurotmesis + repair by coaxially-aligned skeletal muscle autograft

Much of the work performed at EPNRL and its predecessors in London and Cambridge between 1985 and 1995 involved the assessment of the coaxially aligned freeze-thawed muscle autograft FTMG (Gattuso, Glasby, Gschmeissner, & Norris 1989; Glasby et al. 1986a; Glasby, Gschmeissner, Huang, & De Souza 1986d; Glasby, Gschmeissner, Hitchcock, & Huang 1986c; Glasby 1990; Glasby 1991; Glasby, Gattuso, & Huang 1988; Glasby, Hems, & Pell 1992; Norris et al. 1988; Pereira et al. 1991). This is essentially an orientated matrix of muscle basement membrane tubes which can serve as an aligned growth-pathway for pioneering axons. As it does not contain Schwann cells the potential for using this sort of graft over long distances is small. However for gaps of up to five centimetres a good degree of success has been

evinced *provided that* the correct procedure is followed in preparing the graft. Poor reports of its performance have emerged in the literature and this is entirely unsurprising when one examines the unorthodox methods used by some workers (Calder & Norris 1991). The excised piece of muscle should be wrapped in foil to protect it from the direct action of the coolant and then placed in liquid nitrogen (–196°C) until completely frozen. After this thermal shock it is subjected to an osmotic shock by thawing in distilled water. The result is that the basement membrane tubes persist but their contained sarcoplasm is disrupted and is rapidly phagocytized after implantation. This is carried out by interposing the trimmed muscle block into the nerve gap and attaching it to the epineurium of the nerve with interrupted 10/0 polyamide sutures. Over small distances this technique can obviate the need to take donor nerves and is thus especially useful in small children. The technique has also been shown to be of benefit in the treatment of painful neuromata (Thomas et al. 1994).

EXPERIMENTAL VALIDATION OF THE SHEEP MODEL

The experiments recorded hereafter were devised and carried out with a view to the assessment, at various times, of diagnostic techniques and methods for the surgical repair of nerves, using the sheep model. Taken together, these experiments represent a corpus of information which may be analysed to provide an evaluation of the model itself. That the sheep is a useful and amenable model for the surgical repair of peripheral nerves in the human is the hypothesis to be tested by this part of the work.

CHAPTER 3 — METHODS OF ASSESSMENT

Quod enim mavult homo verum esse, id potius credit.

(Francis Bacon, *Novum Organon*)

BACON'S observation that we believe what we want to believe stresses the necessity for controlled scientific tests and their statistical analysis. In the study of peripheral nerve injuries, including their diagnosis and follow-up, we may identify three broad groups of tests which are available. These are:

1. Tests which are readily usable in clinical practice
2. Tests which are potentially usable in clinical practice
3. Tests which are confined to the laboratory

For the purposes of this study the first of these groups includes those tests which are already commonly in use in so far as the inclusion of nerve conduction studies in the management of peripheral nerve conditions may ever be called 'common'[15]. The important point about this group is that the tests should be useful, cost effective and minimally invasive.

The second group is the most controversial and, it is hoped, some of this controversy may be laid to rest by the work presented here. There is no doubt that current electrophysiological investigation and management of peripheral nerve conditions could be improved by the application of more sensitive and discerning tests. The second group contains examples of these whose elevation to group 1 may be impeded by their complexity, invasiveness, duration or cost.

[15] At a recent joint French/British conference a show of hands indicated that >80% of French surgeons and <20% of British surgeons 'require' nerve conduction studies in order to confirm the diagnosis of carpal tunnel syndrome. This disparity is interesting. In Edinburgh at the present time, the waiting-list for nerve conduction studies is longer than that for the operation.

The third group of tests invites no controversy. These are often tests which require direct surgical access to the repaired nerve or even their removal for histological study. As such they are beyond consideration for clinical management but in the laboratory they may be the most informative. They have been the mainstay of much experimental work for many years. The value of these tests to the clinician is that they allow the setting of absolute standards and define practices which may be expected to improve outcome in the clinic.

It should be noted that no comment is made here about tests for sensory nerve function. With the exception of those involved in the estimation of receptive-field area (Findlater, Reichert, & Glasby 1990; Fullarton & Glasby 1997; Reichert , Findlater, & Glasby 1998) these tests involve communication with the patient and this is impossible with animal subjects. It is this very communication which introduces a subjective element into such tests and this may, to a varying extent, confound them. A considered treatise on their use in the upper limb is that of Tubiana (Tubiana, Thomine, & Mackin 1996). There is no doubt that they could be the mainstay of assessment in clinical practice as they have much to offer. Unfortunately for reasons of speed and economy their application is often in truncated form and this greatly impairs their objectivity and value. This must be all too often said of neurological examination in general and is one of the great 'false-economies' of clinical practice.

TESTS READILY USABLE IN CLINICAL PRACTICE

The tests in this group are, in clinical practice, generally taken together and called 'Nerve Conduction Studies' (NCS). For assessment of motor systems they may be taken to include:

- Latency of the CMAP.

- Maximum (motor) conduction velocity (CV_{max}).
- M-wave amplitude
- Minimum F-wave latency (F_{min}).

Latency of the CMAP.

This measurement is made as part of the process of obtaining CV_{max}. The measurement is made from the stimulus artifact to the take-off of the CMAP from the isoelectric line[16]. Poor technique can lead to widely differing results so it is imperative that a good (as near as possible to 90°) take-off is identified. This corresponds to a positioning of the recording cathode over the 'motor point' of the muscle and repays sedulous attention to detail. In some instances it may be more logical to tabulate results in terms of latency rather than velocity as, for example when considering a multineuronal pathway. Also, latency may be used for comparison with, for example, other indices measured using the dimensions of time such as central motor latency and F-wave latency. The technique being part of that used to measure velocity, is considered below.

Maximum (motor) conduction velocity CV_{max}.

Segmental CV_{max} is measured using two stimulating electrodes and recording the CMAP. It reflects only the velocity of the fastest conducting axons which in a motor nerve accounts for about 5% of the total population of motor neurons (Dorfman, Cummins, & Abraham 1982). Nevertheless, measurement of CV_{max}, in clinical practice, remains the single most useful objective test of function. This is partly

[16] In some laboratory studies it has been conventional to measure latency to the peak of the action potential which, if recorded using an a.c. amplifier, corresponds to dV_{max}/dt. However in clinical practice the take-off point is now always used.

because many disease processes either affect all nerve fibres uniformly or predominantly affect the faster fibres.

Two types of study have been used to investigate the factors which affect nerve conduction. Experimental studies have the advantage of providing data derived directly from nerve fibres but the disadvantage of covariance among some of the variables, for example, fibre diameter and myelin sheath thickness. Theoretical studies involve computer simulations by means of mathematical models and have the advantage of permitting a consideration of the different parameters which may affect the variables being measured. The disadvantage of this type of study is that it is based on a set of assumptions, often derived from studies of submammalian species.

Factors affecting nerve conduction

Certain physical and chemical factors in the nerve fibres themselves and in the milieu in which they exist affect their conducting properties. Principal among these are:

Fibre Diameter

Several experimental studies have shown that for myelinated fibres, CV_{max} is virtually proportional to fibre diameter (Waxman 1980). Hursh has shown in the cat that the maximum velocity of conduction for a nerve can be predicted by multiplying the diameter of the largest fibre (in μm) by 6 (Hursh 1939). Also in the cat, Boyd found that the ratio between fibre diameter and conduction velocity was lower for γ fibres than for α fibres. The multiplication factors he derived for conversion of fibre diameter (μm) to conduction velocity (m s^{-1}) were 5.6 for α-fibres and 4.4–4.5 for γ fibres (Boyd 1964). Although these are lower than those of Hursh the general argument that for geometrically similar fibres CV_{max} is proportional to fibre diameter

appears to hold. This has been supported by computer models (Goldman & Albus 1968; Rushton 1951).

Myelin Sheath Thickness

The ratio of axon diameter to fibre diameter is termed the 'g-ratio'. The g-ratio is thus an expression of myelin sheath thickness for a given axon diameter. Conduction velocity is affected by myelin sheath thickness (Smith & Koles 1970) and there is general agreement that conduction velocity is optimized at a g-ratio between 0.6 and 0.7 (Goldman & Albus 1968; Kolcs & Rasminsky 1972; Moore et al. 1978; Rushton 1951; Smith & Koles 1970).This value is generally observed experimentally in normal peripheral nerves but the relationship may have been oversimplified: Brill et al have also implicated myelin sheath capacitance as well as a determinant of conduction velocity (Brill et al. 1977).

Internodal Length

In the cat Coppin and Jack demonstrated a non-linear relationship between internodal length and conduction velocity (Coppin & Jack 1971). The relationship was found to be linear on a semi-logarithmic plot and Goldman and Albus suggested there was an optimal internodal length for a given fibre diameter and that this length was usually to be observed in normal peripheral nerves (Goldman & Albus 1968). Brill (*op cit*) used computer simulation techniques to demonstrate the optimal internodal length to be a ratio of mean internodal length to mean axon diameter of between 100 and 200. Fibres with internodal lengths around the normal range of 1000–2000μm range were shown to be relatively insensitive to changes in internodal length as there was optimal conduction over a broad range of values. After regeneration a reduction in internodal length did not affect conduction velocity until it had been reduced to less than half its

normal value. However as such a reduction is quite common after neurotmesis injuries the correlation between these two variables has been found to be most useful (Gattuso 1988; Gattuso, Glasby, & Gschmeissner 1988).

Age

In newborn human infants motor conduction velocity is around 27m s^{-1} (Johnson & Olsen 1960). A study on human ulnar nerves showed the highest conduction velocities in young adults but these values could be achieved at the age of four to five years (Wagman & Lesse 1952). In the same study it was also found that conduction velocity decreased after the age of sixty. The authors suggested this to be a consequence of selective degeneration of the fastest fibres, decreased oxygen supply with consequent slower metabolism in neurons or lower temperature of the nerves in the forearm. Norris *et al* found that conduction velocity decreases with age but they suggested that this was more likely to be due to vascular changes in the nerve trunk and to altered metabolism which resulted in changes in membrane properties rather than to selective degeneration of fibres or to an altered temperature gradient (Norris & Wagman 1953).

Immediate environment

Several experimental studies have shown a linear relationship between conduction velocity and temperature. Johnson and Olsen described a change in conduction velocity of 5% per °C (Johnson & Olsen 1960). Other studies have shown a decrease in motor conduction velocity of 2.4m s^{-1} per °C between 38°C and 29°C (de Jesus, Hausmanowa-Petrusewicz, & Barchi 1973). De Jesus *et al* (*op* cit) examined the relationship between human limb temperature and conduction velocity and found a Q_{10} of 1.51 for both sensory and motor fibres. Computer simulations also confirm an increase in conduction velocity with an increase in temperature (Moore, Joyner, Brill,

Waxman, & Najar-Joa 1978). Other factors in the environment surrounding a nerve can also affect conduction velocity. For example, phenytoin therapy and haemodialysis can transiently affect conduction velocity (Waxman 1980).

Measurement of Maximum Conduction Velocity

The delay (latency) measured between the time a motor nerve is stimulated and the take-off of its corresponding CMAP consists of three components: the conduction time from stimulating cathode to the nerve terminal, the time for transmission across the synaptic cleft and the time for muscle excitation. Segmental velocity, confined to a specified length of nerve nerve, is measured by stimulating the nerve at two different points and subtracting the resulting latencies. This cancels out the latter two components which would be the same in both cases. By measuring the distance between the two cathodes the maximum conduction velocity for that segment between the cathodes can be calculated.

Experimental Set-up

The general approach to and exposure of the median nerve has been outlined in Chapter 2. The unipolar ground electrode was placed on the right lateral chest wall and the surface recording electrodes were placed over flexor carpi radialis (FCR), the cathode over the motor point and the reference electrode distally over the tendon. These electrodes each consisted of an $Ag/AgCl^-$ disc; they were carefully positioned only after scrupulous cleansing of the skin and each was filled with electrode conducting gel. Bipolar platinum wire stimulating electrodes were positioned on the nerve proximal and distal to the site of injury and held in place with Leyla retractors mounted securely on a bracket connected either to the head frame or to the side-bars of the operating table.

The Medelec machine was set up initially with a sweep speed of 20ms FSD, and a gain of 2mV cm^{-1}. These settings were altered if necessary to obtain an optimal trace. The nerve was stimulated using square wave impulses of 50μs duration at a frequency of 1Hz. Constant-current stimulation was used because in this situation it provides the most constant stimulus (Kimura 2001). The two sites of stimulation may be termed S1 and S2.

The amplitude of the current was gradually increased until the target muscle was noted to be twitching and a CMAP, (M1), was observed on the screen. The intensity of the current was increased further until the amplitude of M1 no longer increased i.e. the maximal stimulation current had been reached. The gain for further stimulation was then increased by 30% and this value noted as the supramaximal stimulating current. A supramaximal current of this size must be used to ensure the activation of all axons innervating the muscle (Kimura 2001). It has been shown that while large diameter, fast fibres have a lower threshold for excitation than thinner, slower fibres these latter are only recruited at relatively high stimulus levels (Borg 1980; Cummins & Dorfman 1981a). The same procedure was carried out with the distal stimulating electrode (S2), and its resultant CMAP (M2) was recorded.

Then:

$$CV_{\max} = \frac{(S1 - S2)}{(M1 - M2)}$$

Where:

($S1$-$S2$) = distance between stimulating electrodes (mm).

($M1$-$M2$) = difference in M-wave latencies (ms).

While it is important to appreciate that in order to obtain a useful measurement of CV_{max} all of the fibres contained in the nerve should be activated, the figure which is obtained corresponds merely to the fastest group of activated fibres. As these fibres

never recover completely after nerve transaction and injury the value of CV_{max} obtained from a repaired nerve must represent a different population of fibres from that which it would be ideal to have regenerated (Cragg & Thomas 1961; Cragg & Thomas 1964; Glasby, Gattuso, & Huang 1988). This problem appears to be more theoretical than actual if we look to present clinical use. It would be fair to say that measurement of CV_{max} remains the one objective test used universally though almost certainly not often enough. A more precise and therefore useful theoretical measurement would be the entire range of (altered) velocities specified by the different sized populations of nerve fibres. The computed value CV_{Dist} goes some way to achieving this and will be discussed later.

M-wave amplitude

M-wave amplitude may be measured as a by-product of CV_{max} estimation. It is only useful if one is sure that supramaximal stimulation was carried out. The choice of a stimulus amplitude 30% above maximum should ensure this. There has been much debate about how far useful information is to be gained from this variable (Binnie et al. 1995; Kimura 2001; Lenihan 2001). Its principal role is in the rule-of-thumb approximation that in neurapraxia, conduction is slowed without a significant fall in M-wave amplitude whereas in axonotmesis both CV_{max} and M-wave amplitude are reduced.

Minimum F-wave latency.

This is an extremely important electrophysiological investigation because it allows access to the proximal segment of the motor conduction pathway. It may therefore be the only test available where the injury is very proximal. F-waves result from the antidromically activated 'backfiring' of anterior horn cells. An F-wave is, therefore, a

compound action potential evoked intermittently from a muscle by a supramaximal electrical stimulus to a nerve (Delisa et al. 1994). The intermittent qualities of the F-wave are significant. The F-wave is elicited in approximately 1 to 5% of antidromically activated motor neurons (Kimura 2001). F-waves vary in latency and waveform. The inherent variability of the latency and its configuration makes the use of F-wave less precise than that of the direct CMAP. Compared with the maximal M wave of the same muscle, the F-wave has a smaller amplitude (1 - 5% of the M wave). The latency of the F-wave is longer with more distal sites of stimulation. This indicates that the impulse destined to elicit the F-wave travels away from the recording electrode toward the spinal cord before it returns to activate distal muscles. The few millisecond interval between the earliest and latest F-wave results from transmission in fast and slow conducting fibres respectively (Peioglou-Harmoussi et al. 1985b). By convention the minimum F-wave latency F_{min} is used for diagnostic purposes as it shows the least variability.

The amplitude and frequency (of incidence) of the F-wave provide a measure of motor neuron excitability (Fisher, Shahani, & Young 1978). In normal human subjects, F-wave frequency varies, and has a mean of 79% (Peioglou-Harmoussi et al. 1985a) This departure from a frequency of 100% could be due to F-waves discharged from hyperexcitable cells in the anterior horn, being blocked because the proximal segment of the nerve is still refractory from the antidromic impulse. Conversely, no response may be elicited from antidromic impulses producing subliminal depolarization in hypoexcitable cells (Kimura 2001).

Methods

Recording electrode placement is the same as for conventional M-wave recording. The stimulating electrode is usually the more distal of the two used for M-wave

recording. However the polarity must be reversed so that the cathode is proximal in order to avoid anodal blocking. Measuring distance is always difficult as this must be twice the distance between the stimulating cathode and the spinal cord plus the short distance between the stimulating cathode and the recording cathode. It is usually impossible to measure this accurately so that latencies rather than velocities should be used in considering F-wave effects. All stimulations must be supramaximal. An optimal display of F-waves requires a sensitivity of 100 to 500μV, with a sweep speed of 10 to 20ms cm^{-1} and a stimulation rate of 2 Hz.

These settings truncate and compress the simultaneously recorded M wave into the initial portion of the trace. F-wave latencies vary by a few milliseconds from one stimulus to the next, so it is necessary to sample more than ten F-waves for a study to be regarded as adequate (Kimura 2001). F_{min} in the present study was found by applying supramaximal stimulations until twenty traces had been acquired and stored. The EMG machine allowed for automatic shifting of successive sweeps vertically. Each trace was then inspected separately for the presence of an F-wave. The latency and amplitude of the identified F-waves were marked automatically using the appropriate analytical algorithm of the Medelec machine. The maximum, minimum and mean values for both latency and amplitude were digitally displayed on the EMG screen and recorded.

Care was taken to identify and reject other causes of late responses such as the A-wave. The A-wave is a compound potential evoked consistently from a muscle by submaximal electric stimuli to the nerve and frequently abolished by supramaximal stimulation. (Delisa, Lee, Baran, Lai, Spielholz, & MacKenzie 1994) The amplitude is similar to that of the F-wave but the latency is more constant. The A-wave usually

appears between the M wave and F-wave (King & Ashby 1976). It is due to normal or pathological branching (Kimura 2001).

Clinical uses of F-wave measurement

Since the F-wave is served by α-motor fibres in both the afferent and efferent limbs of its pathway its study is of clinical value in assessing the integrity of these fibres and gives information regarding conduction in proximal as well as distal portions of peripheral nerves (Lenman & Ritchie 1987).

The F-wave latency may be prolonged in patients with peripheral neuropathy as well as in patients with root lesions and nerve entrapment. In the Guillain-Barré syndrome where the proximal segments of the nerves may be affected, prolongation of F-wave latency may be evident before there is slowing of the peripheral nerve conduction velocity (Shahani, Potts, & Domingue 1980).

Central motor conduction time (*CMCT*)

The F-wave is also of value in calculating approximate values of the latency of the total peripheral component (*P*) of the motor pathway. It is impossible to measure this by conventional means because one is unable to stimulate the α-motoneurones at their cell bodies. Stimulation of the whole spinal cord by means of a magnetic stimulator is a possible alternative but these instruments are very imprecise in targeting particular sites (Drew 2002) . By assuming a value of 1ms for the turn-around time at the α-motoneuron cell body the total peripheral component (*P*) of the central motor conduction time may be calculated thus:

$$P = ½ (F + M - 1)$$

Where:

P = peripheral latency of motor pathway (ms)

F = minimum F-wave latency (ms)

M = M-wave latency (ms)

If the value of P is known, the latency of the upper motoneurone pathway (*CMCT*) may be calculated if the cortex is stimulated either electrically or by the use of a magnetic coil and the time to the distal CMAP recorded.

If the total conduction time is T, then:

$$CMCT = T - P$$

This (*CMCT*) is conventionally termed the central motor conduction time (Binnie, Cooper, Fowler, Mauguière, & Prior 1995).

TESTS POTENTIALLY USABLE IN CLINICAL PRACTICE

Tests may be categorized in this group because they are complicated to carry out, invasive to a degree which might make their clinical use questionable, expensive or time-consuming. In most of these there is a case to be made for use of the test provided that it adds significantly to the strategy of management. Needless to say, this matter is controversial in respect of the tests considered here:

- Jitter
- Refractory period
- Distribution of conduction velocities CV_{Dist}

Jitter

Single fibre electromyography (SFEMG) was developed to enable assessment of the physiology of individual motor units (Stålberg & Trontelj 1994b). If a nerve fibre is voluntarily activated or stimulated supramaximally and single fibre action potentials (SFAPs) are recorded from a target muscle fibre, there is a variability in the latency of the SFAP termed *jitter*. The precise cause of *jitter* is uncertain but it may be

due to variation in the transmission-time of the impulse along the nerve fibre, variation in transmission-time across the neuromuscular junction (NMJ) and/or variation in transmission-time along the muscle fibre.

Types of jitter

Jitter may be voluntarily activated (VAJ) or stimulated transcutaneously (TSJ). In VAJ it is measured in voluntarily activated muscle whilst in TSJ a single motor axon is electrically stimulated. In VAJ SFAPs are recorded from a pair of muscle fibres innervated by the same motor axon. One of the SFAPs is used as a time reference to trigger recording and jitter is determined by the variability in the time (interpotential interval) between the two SFAPs. In TSJ a single terminal motor axon is stimulated and a SFAP recorded from a single muscle fibre the time-datum here being the stimulus artefact. In this case jitter is calculated from the variability between successive action potentials. In a normal setting TSJ has a lower value than value than VAJ such that: $TSJ = \sqrt{2}(VAJ)$; (Stålberg 1990; Stålberg & Trontelj 1994b; Trontelj, Stålberg, & Mihelin 1990).

Voluntary jitter requires the co-operation of the patient whereas TSJ does not. TSJ can therefore be useful for young children although it requires the use of three needles rather than a single recording SFEMG needle. TSJ is likewise of value in the unconscious and in those with profound muscle weakness and is obviously the only method available for animal experiments.

In VAJ voluntary contraction results in a low strength of activation of the target muscle and thus in the recruitment of low-threshold, small motor units, consisting mainly of Type I fibres (Trontelj, Stålberg, & Mihelin 1990). This technique can be used to assess the order of physiological recruitment of motor units, their dependence on afferent input and their relation to fatigue (Trontelj et al. 1992; Trontelj & Stålberg

1992). In jitter measurement the geometry of the relationship between nerve and muscle appears to determine the characteristics of activation. Large axons have lower activation thresholds than smaller ones (Borg 1980), but because of the high electrical impedance of muscle, the distance of the activated axon from the stimulating electrode is thought to be more important than the properties of the axon itself in determining which muscle fibres become excited. In TSJ therefore, in contrast to VAJ, both low and high threshold motor units are recruited but predominantly Type II muscle fibres (Trontelj et al. 1986; Trontelj, Stålberg, Mihelin, & Khuraibet 1992; Trontelj & Stålberg 1992; Trontelj, Stålberg, & Mihelin 1990).

An important concept to be considered in jitter studies is the effect of the velocity recovery function (VRF): this effect is seen particularly in voluntary jitter. VRF is a phenomenon whereby the conduction velocity of muscle fibres is affected by a conditioning impulse (Mihelin, Trontelj, & Stålberg 1991), so that after the passage of such an impulse there is an initial period of subnormal conduction velocity followed by a period of supranormal conduction. The amplitude of the SFAP may also be reduced after a discharge. In a normal muscle fibre the conduction velocity may vary by 0.5–1% for consecutive discharges (Stålberg & Trontelj 1994a). In pathological conditions the effect of VRF may be much higher. However in the experimental situation VRF has been found to have no overall effect in the calculation of stimulated jitter because the same muscle fibre is stimulated at a constant rate (Trontelj, Mihelin, Fernandez, & Stålberg 1986). TSJ may therefore be preferable to VAJ in those situations where there may be a large VRF effect.

Magnitude of jitter

Since there is little variability in the conduction time of normal nerve and muscle fibres (Stålberg & Trontelj 1994a; Stålberg & Trontelj 1994b), it is reasonable to

suppose that, in a normal setting, the main source of jitter is events taking place at the neuromuscular junction. In particular this is thought to be electrical membrane noise which causes a fluctuation in the threshold for depolarization of the muscle membrane and a variation in the amplitude and rate of rise of the end-plate potential.

Abnormally low levels of jitter may be observed in VAJ jitter when a branching muscle fibre is activated since no end-plate transmission is necessary between the triggering impulse and the second SFAP. This phenomenon is occasionally seen in normal muscle but more commonly in association with muscular dystrophies. In TSJ direct stimulation of muscle fibres may result in abnormally low (≤4μs) jitter values (Stålberg 1990). This cut-off value is the level of jitter associated with the normal NMJ.

TSJ may be over-estimated by failure to use supramaximal stimulation which can result in a variable rate of stimulation inducing 'myogenic' jitter by the mechanism of VRF in muscle fibres. Transmission at the NMJ is reduced at stimulation rates less than 2Hz whereas the amplitude of SFAPs may decrease at stimulation rates above 20Hz (Trontelj, Stålberg, Mihelin, & Khuraibet 1992; Trontelj & Stålberg 1992). Superimposition of two SFAPs can result in erroneously high jitter measurements if the interfering peak has an innately higher jitter value or is not stimulated supramaximally (Stålberg *op cit*). Abnormally high levels of jitter are associated with pathological processes involving nerve conduction, NMJ transmission and muscle fibre conduction. TSJ studies have found abnormally high levels of jitter after peripheral nerve injuries (Lenihan et al. 1997; Lenihan, Sojitra, & Glasby 1998).

Experimental measurement of TSJ

In TSJ a single terminal motor axon is stimulated and action potentials recorded from a single muscle fibre using a single-fibre (SFEMG) needle. This consists of an inner thin platinum or silver wire cathode and an outer steel reference anode, separated by a layer of insulation. The cathode is exposed, through a window in the casing, 1mm to 5mm proximal to the needle's tip and opposite to the bevel. This is supposed to avoid recording from muscle fibres which may be damaged by insertion of the needle.

Two unipolar needle electrodes inserted into the muscle in question are used to stimulate a terminal motor axon one hundred times using constant-current square-wave pulses of 10Hz frequency and 50μs duration. The amplitude is supramaximal, its value is determined as described above. The latencies of the resulting action potentials from an associated muscle fibre are recorded using SFEMG electrode. Recording is triggered on the rising edge of the stimulus. Each measurement of latency is termed an interpotential interval (IPI). The variability of these IPIs is the TSJ value or mean consecutive difference (MCD) and is calculated using the following equation:

$$MCD = \sum \left(\frac{(IPI_n) - (IPI_{n-1})}{n} \right)$$

Where:

MCD = mean consecutive difference (μs)

IPI = interpotential interval (ms)

n = number of stimuli

This process is repeated twenty times, with different sitings of the recording electrode in the muscle in order to give a reliable result (Stålberg & Trontelj 1994b). These are averaged to give a mean TSJ value for the muscle.

Experimental Set-up for the Median Nerve

A Medelec Synergy machine (Oxford Instruments, Surrey, UK) was used for all stimulating and recording procedures in the measurement of TSJ.

The animal was positioned and the skin prepared as described in Chapter 2. The ground electrode was placed on the lateral wall of the right side of the chest of the animal and connected to the preamplifier. Two unipolar stimulating needle electrodes were inserted into flexor carpi radialis (FCR) which is supplied by the median nerve. The cathode was placed at the previously-determined motor point of FCR and the anode (reference) electrode was sited 0.5cm proximal to this. The muscle was stimulated with constant current pulses of 10Hz frequency and 50μs duration. The bandwidth for recording was set at 500Hz – 10kHz.

The stimulus amplitude was increased until the muscle was seen to be twitching. An SFEMG recording needle electrode was inserted into the area of the FCR muscle seen to be contracting maximally. The stimulus amplitude was increased until there was no further reduction in latency: this was between 2mA and 3mA. The recording electrode was then manipulated by rotating it to obtain sharp muscle fibre SFAPs on the monitor screen. It was held in this position and one hundred SFAPs were recorded. The TSJ value was calculated by the Medelec using an algorithm using the above equation. The electrode was repositioned after every three sets of recordings. Twenty sets of recordings were made and a mean TSJ value for the muscle was calculated.

Refractory period

Measurement of the absolute and relative refractory periods of nerves after injury and repair provides a means of differentiating their conducting properties. The measurement of the excitability of a nerve is a useful and important way of

determining its properties which are preserved for a short time even after death (McDowall K.L. et al. 1998; Straton, Busuttil, & Glasby 1992).

Immediately after depolarization an axon becomes inexcitable. This is called the absolute refractory period (ARP) and lasts between 0.5ms to 1.0ms in a normal nerve (Kimura 2001). The ARP is a result of closure of sodium channels, to allow repolarization of the axon. Initially these channels are unable to reopen regardless of the magnitude of the stimulus, but during the subsequent few milliseconds an extremely large stimulus, beyond physiological levels, can trigger depolarization: this is permitted during the relative refractory period (RRP). During the RRP impulses propagate more slowly than normally because of the increased time required for each cell to generate an action potential. In human volunteers, Gilliatt demonstrated the slower conduction velocity associated with the RRP (Gilliatt R.W. 1963) by applying two supramaximal stimuli to the median nerve at the wrist and recording CNAPs from the nerve at the elbow. With ISIs of 5ms and 3ms the interval between the CNAPs at the elbow was the same as the ISI. However, for ISIs less than 3ms the interval between the APs was greater than the ISI as the second impulse occurred during the RRP of the nerve and was therefore conducted more slowly.

In 1976 Kimura described a collision technique to measure refractory period in motor nerves (Kimura 1976). This entailed the use of two proximal stimuli and a single distal stimulus; compound muscle action potentials (CMAPs) were recorded from a target muscle. The first proximal stimulus and the distal stimulus were delivered simultaneously to produce an early CMAP by the orthodromic route from the distal stimulus, whilst the antidromic impulse from the distal stimulus collided with the orthodromic stimulus from the first proximal stimulus and thus did not produce a CMAP. The interstimulus interval (ISI) between the proximal impulses was gradually

increased and as the orthodromic impulse from the first proximal stimulus was blocked there was no distortion of the CMAP associated with the second proximal impulse, as had been encountered with earlier methods using only two stimuli.

With very short ISIs between the proximal stimuli only those fibres with a short refractory period recovered from the conditioning impulse and were able to conduct a second impulse and generate a late (test) CMAP. As the ISI was further increased, successive populations of fibres were recruited and the test CMAP increased in amplitude. Therefore, the fastest-recovering fibres generated the first increase in the test CMAP and the slowest-recovering fibres contributed the final increment.

Kimura *et al* (*ibid*) suggested that this method could be used to define the range of absolute refractory periods. He ascribed a decrease in amplitude of the test CMAP to the failure of activation of fibres at the site of the second proximal stimulus because these fibres were still refractory. It followed that the amplitude of the test CMAP was proportional to the number of fibres no longer refractory and that if the amplitude of the test CMAP were compared to the amplitude of the proximal stimulus fired in isolation (i.e. where all the fibres would be active) this would give the proportion of the fibres recovered from the refractory period.

Kimura *et al* (*ibid*) suggested that an increase in amplitude of the test CMAP was attributable to the ARP as once a fibre was activated, the impulse would be propagated to the muscle regardless of the length of the refractory segment. They also proposed that any change in latency of the test CMAP would be the result of the recovery of conduction velocity of the fastest fibres and hence a measure of the relative refractory period of those fibres.

Ingram *et al* described an alternative technique to measure the range of refractory period in motor fibres using two proximal shocks and two distal shocks (Ingram,

Davis, & Swash 1986). The ISI between the proximal stimuli was kept the same and was longer than the refractory period of the whole nerve. Therefore the second proximal stimulus was always conducted. The ISI between the distal stimuli was varied. The first proximal shock and the first distal shock were fired simultaneously so that the orthodromic impulse from the proximal stimulus was always blocked. When the ISI between the distal stimuli was less than the shortest refractory period, the second distal stimulus did not elicit a response and this allowed all the fibres contributing to the orthodromic impulse from the second proximal impulse to generate a late CMAP which was therefore of maximum size. As the distal ISI increased, successive populations of fibres were recruited and these recruited impulses collided with the second proximal impulse and blocked some of the action potentials. The result of this collision was a reduction in the size of the late CMAP. As the distal ISI was increased further, progressively more of the second proximal orthodromic impulse was blocked until the second distal impulse completely blocked the second proximal impulse and no late CMAP was generated. Subsequent disappearance of the late CMAP marked the end of the range of the refractory periods. Ingram *et al* preferred their method to Kimura's because, as the ISI between the proximal stimuli was constant, the effect of the subnormal period of nerve conduction and the VRF effect of muscle would have been the same in all case and the indication of the end-point of the range of refractory period by the disappearance of the late CMAP was easier to identify.

In 1976 Betts *et al* described a method to measure refractory period using double stimulation with an 'automatic subtraction technique' (Betts, Johnston, & Brown 1976) which effectively cancelled the conditioning response, thus isolating the test response. Kopec et al described a similar technique to that of Betts et al but recorded

CMAPs from muscle (Kopec, Delbecke, & McComas 1978). In human volunteers they stimulated the median nerve at the elbow or at the wrist and recorded CMAPs from the thenar eminence. The CMAP generated by a single supramaximal stimulus was stored digitally as a reference trace. Paired stimuli were then applied with a range of ISIs. The CMAPs (conditioning and test traces) were also stored and then the reference trace was subtracted digitally. Since the CMAP from the single stimulus was the same as the conditioning CMAP this isolated the test CMAP. The amplitude of this CMAP was then measured and used to calculate the proportion of fibres in the refractory period. The absolute refractory period was determined from the shortest ISI which allowed generation of a test CMAP.

In the present study the minimum absolute refractory period (ARP_{min}) and the maximum absolute refractory period (ARP_{max}) were calculated using the paired shock technique described by Kopec et al (Kopec, Delbecke, & McComas 1978). A reference trace was generated using an ISI = 0ms. Paired stimuli were then applied to the nerve over a range of ISIs from 0ms to 10ms. Test traces were isolated by digitally subtracting the reference trace from the subsequent traces. ARP_{max} was defined as the ISI associated with the first observable decrement in amplitude of the test CMAP. Previous workers have interpreted the first decrement in the amplitude of the test CMAP to represent the relative refractory period (*RRP*) however, in the *RRP* there is slowing of impulse conduction but no blocking and therefore a decrease in amplitude of the test CMAP must result from fibres in the *ARP*. ARP_{min} was defined as the smallest ISI before the disappearance of the test CMAP. The *RRP* was not assessed in this work as it is so variable as to be of little practical use.

Experimental measurement of refractory period

The positioning of the stimulating and recording electrodes on the nerve and muscle was identical to that used in the measurement of CV_{max} and $CV_{Dist.}$. However, in the calculation of refractory period only the distal stimulating electrode, S1, was required. S1 was connected to stimulator A on the Medelec machine, which provided constant current stimulation using square wave pulses of 50μs duration.

The amplitude of the current required to ensure supramaximal stimulation was determined using a single stimulus. The amplitude of the current was gradually increased until the resulting CMAP no longer increased in size. The stimulus was then increased by a further 30% to ensure activation of all axons. This was designated the supramaximal current and was used for all subsequent stimulation of the nerve.

A reference trace was generated by setting the interstimulus interval ISI = 0ms. Using an ISI of 0ms all impulses from the second stimulation were blocked and therefore only one CMAP was generated. This reference trace was stored and later digitally subtracted from the subsequent traces in order to isolate the test trace.

The ISI was then increased to 10ms to obtain two discrete CMAPs. With this ISI the amplitude of the second (test) CMAP was the same as the first (conditioning) CMAP. This indicated that no fibres were blocked and so no fibres were refractory.

The ISI was then decreased in steps of 0.5ms until the amplitude of the second CMAP was seen to decrease. The ISI associated with the first observable decrement in amplitude of the test CMAP was designated ARP_{max}. The decrease in the test CMAP was ascribed to the slowest recovering fibres still within in the absolute refractory period. With the longer ISIs associated with ARP_{max}, the conditioning CMAP did not distort the test CMAP. Therefore it was not necessary to isolate the test CMAP by digitally subtracting the reference trace. However, in those cases where it was difficult

to determine if there was a decrease in the amplitude of the test CMAP, consecutive traces were superimposed on top of each other to compare the amplitudes of the traces.

After determination of ARP_{max} the ISI was then further decreased in steps of 5ms. This produced a decrease in size of the test CMAP and collision of the test CMAP with the conditioning CMAP. As the collision point was approached, the ISI was decreased by smaller steps of 0.1ms. The reference trace was digitally subtracted from these traces to isolate the test CMAP. Adrian defined that ISI which immediately preceeded that ISI causing a complete block of the test trace, as the absolute refractory period of the nerve (Adrian 1921). In the present study this quantity has been designated ARP_{min}.

Distribution of conduction velocities CV_{Dist}

Measurement of the distribution of conduction velocities (CV_{Dist}) in a peripheral nerve is a technique whereby the distribution of the individual conduction velocities of nerve fibres within a population is calculated. The population under consideration may be the total number of nerve fibres in a nerve trunk or fascicle. CV_{Dist} can be measured for unimodal or mixed nerves. It may be regarded as the electrophysiological counterpart of the morphological fibre-diameter distribution-frequency histogram which historically has been regarded as the most useful laboratory investigation in the study of peripheral nerve injury and regeneration.

Using conventional NCS it is only possible to measure the velocity CV_{max} of the faster fibres within a nerve. In motor nerves CV_{max} relates to only 5% of the total number of fibres (Dorfman, Cummins, & Abraham 1982). Notwithstanding this the test has proved remarkably useful in a clinical setting presumably because many affections of peripheral nerves involves the larger fibres predominantly or all fibres uniformly. However, some disease processes e.g. diabetes mellitus, amyloid polyneuropathy,

hereditary sensory neuropathy, affect nerve fibres non-uniformly and in these anabnormal CV_{Dist} profile is seen when CV_{max} remains within normal limits (Brown, Martin, & Asbury 1974); (Cummins & Dorfman 1981; Dorfman 1984).

Techniques for the derivation of CV_{Dist}

There are several approaches which have been used to find CV_{Dist}: the three most successful of these methods are discussed hereafter.

1.Deconstruction of reconstructed compound action potentials

Dorfmann, the exponent of this method of calculation considers the compound muscle action potential (CMAP) to be the weighted sum of its component motor unit action potentials (MUAPs) (Dorfman 1984). The size and shape of the CMAP is therefore determined by the amplitude and duration of these constituent MUAPs, the number of motor units, the conduction velocities of the nerve fibres and the distance the impulse travels along the nerve.

A CMAP may be reconstructed using a nominal MUAP and histological data regarding the diameters of the relevant nerve fibres. This method depends on assumptions of the relationship between conduction velocity and fibre diameter, the conduction velocity and MUAP spike duration and amplitude. These relationships are defined as weighting functions.

For myelinated nerve fibres conduction velocity is almost linearly related to fibre diameter (Boyd 1964; Hursh 1939). Larger nerve fibres generate shorter MUAPs with shorter latencies whereas smaller fibres generate more, broader MUAPs with longer latencies. The amount of dispersion of the CMAP will increase with a longer conduction distance as the slower fibres progressively lag behind the faster ones. The

amplitude of the action potential is dependant on both the conduction velocity and the number of fibres contributing to it.

The value of CMAP reconstruction was recognized and the earliest experiments were performed as long ago as 1927 by Erlanger and Gasser in 1927 (Erlanger 1927; Erlanger & Gasser 1924; Gasser & Erlanger 1927). Gasser and Grundfest (Gasser & Grundfest 1939) in the cat saphenous nerve demonstrated that a compound nerve action potential (CNAP), almost identical to the recorded CMAP, could be constructed using a nominal sensory unit action potential (i.e. the action potential recorded from a single nerve fibre, SUAP) and a fibre-diameter histogram of the nerve. To reconstruct a CNAP, a conduction velocity was assigned to fibres of a given size. For larger fibres, a variation of 1μm in the diameter of the fibres in each group was allowed because the amount of dispersion of their SUAPs was small. For smaller fibres each group had variations in fibre diameter of only 0.1μm to 0.25μm because of the greater dispersion of the SUAPs. The conduction time was calculated for each assigned conduction velocity using the conduction distance which had been used in the generation of the recorded CNAP. A vector triangle was drawn for each group imitating the dimensions of a SUAP. The height of each triangle was arrived at by multiplying the mean diameter of the fibres in a particular group by the number of fibres in that group. Each triangle touched the abscissa at a point corresponding to the conduction time. Once all the triangles were drawn they were summated and the resulting envelope-curve compared with the recorded CNAP. This was found to be an accurate method of reconstructing a CNAP.

It is logical to suppose that the reverse of this process (i.e. deconstruction of the CNAP/CMAP) may be performed to generate a CV_{Dist} profile. If the amplitude and waveform of a nominal SUAP are known along with the relative weighting functions

then a CNAP can be deconstructed to enable calculation of its component SUAP latencies and hence conduction velocity (Cummins, Dorfman, & Perkel 1979; Cummins, Perkel, & Dorfman 1978).

Inevitably there has been disagreement about the values of weighting functions. For example, Gasser and Erlanger (Gasser & Erlanger 1927) assumed that the amplitude of the SUAP was proportional to the cross-sectional area whereas Gasser and Grundfest (Gasser & Grundfest 1939) assumed it to be proportional to the diameter of the fibre. In the latter work the duration of the spike was assumed to be constant (0.4ms) but Paintal has since demonstrated that spike-duration in mammalian myelinated fibres is inversely related to conduction velocity (Paintal 1973). Most now accept that conduction velocity in myelinated fibres is proportional to fibre diameter but different values for the precise ratio for this relationship have been suggested. In the cat model Hursh found a conduction velocity to fibre diameter ratio of 6:1, Boyd (also in the cat) found a ratio of 5.6:1 whilst in the peroneal nerve in the rabbit Cragg and Thomas found a ratio of 4.4:1 (Boyd 1964; Cragg & Thomas 1961; Cragg & Thomas 1964; Hursh 1939). These differences may have been due to variations in recording technique or inherent differences between specific nerves or between animal species. Also, Cummins *et al* have developed a mathematical model to allow the use of different weighting functions depending on the physiological data which are available (Cummins, Perkel, & Dorfman 1978).

In human sensory CV_{Dist} studies there is an obvious difficulty in determining a nominal SUAP required for single-fibre recordings; futhermore, CNAPs may be distorted by a low signal to noise ratio (Morita et al. 2002). In motor studies the muscle action potentials which are generated are of much higher amplitude and MUAPs are easily recorded using intramuscular needle electrodes. Nevertheless,

MUAPs vary in amplitude, duration and waveform and change in response to disease processes so this makes determination of a nominal MUAP also difficult. Lee et al attempted to circumvent this problem by deriving a 'representative' MUAP for a particular muscle (Lee et al. 1975). An intramuscular bipolar needle electrode was introduced into the thenar eminence of human volunteers and used to identify a spike from a single motor unit and to trigger coincidental recording from two surface electrodes. The signal from the surface electrodes was averaged and the process was repeated for between 100 to 500 discharges of each motor unit to enable determination of a single MUAP as the average of all the recorded discharges. This process was repeated for a further 20 to 30 motor units and all the results were then averaged to give a representative MUAP for the muscle. Using computer simulations it was possible to study the effect of different distributions of conduction velocity on the shape of the CMAP and to determine ranges of conduction velocities associated with particular pathological CMAPs.

2.Comparison of two CMAPs.

In order to derive CV_{Dist} by this method, the shapes of two compound action potentials (CNAP/CMAP), resulting from different conduction segments, are compared. For motor studies, the nerve is stimulated in a manner identical to that for the measurement of CV_{max} (Dorfman 1984). Differences in amplitude and temporal dispersion of the CMAPs are assessed. As the conduction distance lengthens, the amplitude of the associated CMAP decreases and its duration increases. CV_{Dist} is derived as a function of the difference in conduction distance.

This approach has the advantage over the previous method that it does not require knowledge of a nominal SUAP or MUAP. However, the two CNAP/CMAP method may be inapplicable over short conduction distances because of a high noise to signal

ratio (Cummins, Dorfman, & Perkel 1979). Distortion of the signal can result from activation of different nerve fibres at the two stimulation points notwithstanding supramaximal stimulation, branching of nerve fibres especially over long distances and differences in nerve-to-electrode transfer-function (NETF). NETF is determined by the geometry of the nerve with respect to the size and position of the electrode. Wells and Gozani developed a method to normalize NETF using an array of stimulating and recording electrodes positioned along the course of the nerve (Wells & Gozani 1999) and so minimize the level of noise. This allowed shorter segments of nerve to be used. However this method of calculating CV_{Dist} assumes uniform conduction velocity throughout the nerve segment under investigation making it unsuitable for the assessment of localized conduction blocks or discontinuous lesions.

3.Modification of Hopf's Collision technique — Theory

The distribution of motor conduction velocities (CV_{Dist}) within a nerve can be determined using the 'collision technique'. This technique was first developed to assess the minimum conduction velocity within a nerve (Hopf 1963) and uses two supramaximal stimuli applied a known distance apart, at two different locations along the nerve. The stimulus applied at the distal location is called S1 and the stimulus applied at the proximal location is called S2. Initially, S1 and S2 are applied synchronously. S1 produces an orthodromic impulse that results in a compound muscle action potential (CMAP) or M1-wave which is recorded at the target muscle. The S1 stimulus also produces an antidromic impulse travelling in the opposite direction; (this is true also of S2 but irrelevant in the present context). Since S1 and S2 are applied simultaneously, the antidromic impulse from S1 'collides' with the orthodromic impulse from S2 and this prevents S2 from producing a CMAP.

The time interval (interstimulus interval, *ISI*) between the S1 and S2 stimuli is then increased. When the *ISI* just exceeds the conduction time of the fastest fibres plus the refractory period of those fibres, the orthodromic impulse from S2 will begin to produce a second (later) CMAP called the M2-wave. This M2-wave is initially associated with the fastest fibres. The impulses in the slower fibres will still collide with the antridromic impulses from S1 and therefore do not contribute to the size of the M2-wave. As the *ISI* increases further, progressively slower fibres will be recruited. This will result in an increase in the size of the M2-wave as the fibres no longer collide with the antidromic impulse from the S1 stimulus.

The maximum conduction velocity (CV_{max}) must be measured in the usual way before assessing the distribution of conduction velocities. From the CV_{max}, the probable range of conduction velocities can be estimated. This range is divided arbitrarily into equal divisions (40 in the present experiments) beginning with 125% of the CV_{max} (CV_{max} + 10 m s^{-1}, in the present experiments) and ending with 0 m s^{-1}. The value of 0 m s^{-1} was used as the lowest limit to encompass all possible values for CV_{min}. The *ISI* for each conduction velocity was determined using the following formula:

$$ISI = \frac{d}{CV} \qquad \{\text{Equation 1}\}$$

where:

ISI = interstimulus interval (ms)

d = distance between the S1 and S2 electrodes (cm)

CV = conduction velocity at a specific time (m s^{-1})

(In Equation 1, the *ISI* is the conduction time plus the refractory period.)

The refractory periods of the fibres within the nerve are assumed to be inversely proportional to the conduction velocities of the fibres. Failure to incorporate the refractory period would artificially shift the CV_{Dist} to the right. The absolute

refractory period (ARP) of the peripheral nerve is measured (McDowall et al. 1998) and taken to be the refractory period of the fastest fibres. The ARP of the slowest fibres is estimated from the measurement of the relative refractory period for the whole nerve — see above. By the use of these values, a range of refractory periods that is inversely proportional to the conduction velocities is calculated. These values are then used to calculate an adjusted *ISI* (*aISI*). Therefore:

$$aISI = ISI - RP \qquad \{\text{Equation 2}\}$$

where: *aISI* = adjusted interstimulus interval (ms)

ISI = interstimulus interval (ms)

RP = refractory period of a fibre at a given conduction velocity (ms)

And the adjusted conduction velocity (*aCV*) is determined and represented by the following equation:

$$aCV = \frac{d}{aISI} \qquad \{\text{Equation 3}\}$$

where:

aCV = adjusted conduction velocity (m s^{-1})

d = distance between the S1 and S2 electrode (cm)

aISI = adjusted interstimulus interval (ms)

Initially, the largest *ISI* that represents a latency where no impulses are colliding is used. The *ISI* is then reduced to a value that corresponds to 125% of the previously measured maximum conduction velocity. The area of the M2 wave is measured for each *ISI*.

The area of the M2 wave with the greatest *ISI* is considered to be the maximum M2 area. The ratio of each subsequent M2 trace-area to the maximum area is calculated. The relationship between the *aCV* and the ratio of areas is also determined and expressed in the form of a polynomial equation in the general form:

$$f(x) = \frac{a}{b + cx + dx^2 + gx^3 + hx^4 + jx^5 + kx^6} \qquad \{\text{Equation 4}\}$$

where:

$f(x)$ is the ratio of areas

x is the aCV at each ISI

$a,b,\ldots$ are constants

In Equation 4 $f(x)$ represents the rate at which the number of active fibres changes with the change in the quantity aCV, (x). Therefore if the expression on the right-hand side of Equation 4 is differentiated with respect to x, the result ($f'(x)$) represents the relative proportion of active fibres within the nerve at a specific aCV. These data when placed in a histogram yield the CV_{Dist}.

A more complicated but possibly more accurate collision technique was developed by Ingram (Ingram, Davis, & Swash 1987a; Ingram, Davis, & Swash 1987b) using three supramaximal stimuli: a proximal stimulus (p1) fired first, a distal stimulus (d) fired second and a further proximal stimulus (p2) fired last. The ISI between the first and second stimuli is variable ($ISI_{p1\text{-}d}$) whilst the ISI between the second and third stimuli is constant ($ISI_{d\text{-}p2}$). $ISI_{d\text{-}p2}$ is short to ensure collision of all fibres if these stimuli are fired in isolation of the first proximal stimulus. The orthodromic impulse from distal stimulus always reaches the target muscle generating a CMAP (Md). When $ISI_{p1\text{-}d}$ is short, orthodromic impulses from p1 collide with antidromic impulses from d. This allows impulses from p2 to reach the target muscle creating a second late CMAP (Mp2). As $ISI_{p1\text{-}d}$ is increased orthodromic impulses from p1 will pass the distal stimulation site before d is delivered. These impulses will generate a small early MUAP (Mp1). In these fibres the antidromic impulse from the distal stimulus, d, will then collide with the orthodromic impulse from p2 thereby blocking these fibres from

contributing to the late Mp2. The $ISI_{p1\text{-}d}$ which cancels Mp2 is the conduction time and the refractory period for these fibres at the distal site. As $ISI_{p1\text{-}d}$ is incremented the early Mp1, increases and Mp2, decreases in size as more impulses pass the distal stimulation site before the stimulus is fired. The elimination of the Mp2 CMAP corresponds to the conduction time of the slowest fibres CV_{min}.

Ruijten et al compared the methods of Hopf and Ingram in the calculation of motor CV_{Dist} in the peroneal nerves of healthy human volunteers (Ruijten, Sallé, & Kingma 1993). They found no preference for either technique in their study but suggested that Ingram's technique may be superior over shorter distances.

Harayama et al proposed another three-stimuli technique (Harayama et al. 1990). The stimuli are fired in the same order as in Ingram's method (proximal, distal, proximal) but the interval between the first proximal stimulus and the distal stimulus is fixed and the interval between the distal stimulus and the second proximal stimulus is variable. The fixed interval is short to ensure collision of all fibres. The two CMAPs generated correspond to those in Hopf's technique but it is suggested that the second proximal stimulus which generates the second CMAP (M2) is less affected by the refractory period of the nerve because the antidromic impulse from the distal stimulus is cancelled by the orthodromic impulse from the first proximal stimulus and the nerve has longer to recover before it is stimulated again.

A modification of the double-stimulus collision technique was used for the calculation of CV_{Dist} in this work. While acknowledging the potential superiority of the triple-stimulus technique is doubtful if the benefits of the triple-stimulus method are sufficient to overcome the considerable difficulty of acquiring the apparatus to carry out the technique. There is, at present no commercially available EMG/NCS/EP machine which allows independent manipulation of three stimulators: such apparatus

must be specially commissioned. In contrast all of the better commercially available machines are programmable to undertake the double-stimulus technique for no more than the cost of adding a second stimulator if this was not purchased initially. A large ten-channel machine such as may be used for spinal cord intraoperative monitoring will come with two stimulators as standard. Therefore, as the present study was, above all, intended to investigate techniques for clinical use, the double-stimulus technique was adopted. The principal modifications made to Hopf's technique for the present study were the use of modern commercially available computer software for calculation and for curve-fitting and the writing of a custom-made protocol for the Medelec machine. Such modifications are easily within the capabilities of an experienced Clinical Neurophysiologist and it it therefore proposed that the method as described here is appropriate for immediate clinical use.

Measurement of CV_{Dist} by the double-stimulus method

CV_{max} was first measured as described above. For CV_{Dist} the positions of the stimulating and recording electrodes, and their connections to the Medelec machine, were identical to those used for CV_{max}. The distal stimulator, S1, was connected to stimulator A of the Medelec machine and the proximal stimulus, S2, was connected to stimulator B.

The motor point of the target muscle and the supramaximal currents required at both points of stimulation were identified for the measurement of CV_{max} as described previously. Constant current, square-wave impulses of 50μs duration were used. Supramaximal stimulation though always important was especially so in CV_{Dist} as fibres in the slower ranges of conduction have higher thresholds for excitation (Borg 1980; Cummins, Dorfman, & Perkel 1981).

The recording electrodes were manipulated to achieve the optimum CMAPs as the shape of the CMAP affects the pattern of collision. Since the calculation of the area of M2 depends on the accurate placement of markers on the take-off point, turning-points deflection and return to the isoelectric baseline, it is most important that attention is paid to identifying the motor point and having clear waveforms with easily identifiable peaks and troughs.

Initially the same sweep speed and gain selected for the measurement of CV_{max} were used. These were altered later if necessary so that both CMAPs could be seen together on the monitor screen.

The range of ISIs with which the nerve was to be stimulated was then calculated: CV_{max}, which had been measured previously, was used as a guide to the range of conduction velocities to be expected. This range was set from a minimum conduction velocity of 10m s^{-1} to a maximum conduction velocity of (CV_{max} + 10m s^{-1}). These values along with the interstimulus distance, between S1 and S2, were entered into an Excel Microsoft spreadsheet. The spreadsheet had been previously configured to divide this range into forty equal divisions and calculate the corresponding ISI using the following formula:

$$ISI = \frac{d}{CV}$$

Where: ISI = interstimulus interval (ms)

d = distance between stimulating electrodes (cm)

CV = selected conduction velocity (m s^{-1})

These values for ISI were then entered individually into the Medelec machine. An initial ISI of 0ms was selected to generate an M1 reference trace (all impulses from S2

were blocked). The ISI was then increased to the maximum of the previously calculated values and sequentially decremented. At higher ISIs values M1 and M2 were recorded as discrete traces. As the ISI was decreased M2 was seen to collide with M1, decreasing in size until it finally disappeared. The initial reference trace was then digitally subtracted from the subsequent traces to isolate the test trace M2.

On the Medelec screen markers were manually positioned on M2 at the points of take-off, maximum amplitude and return-to-baseline. When these were in place the collision program on the Medelec machine calculated the area of each M2 wave. These areas were then entered into the pre-configured Excel spreadsheet each beside its corresponding (adjusted) conduction velocity (*aCV*).

Both columns of data were then exported into a mathematical modelling program (Datafit, Version 7.1, Oakdale Engineering) where the values for the area of M2 were plotted (as ordinate) against the range of conduction velocities (as abscissa) and the 'best-fit' equation for the data found by means of regression analysis. The programme uses the Levenberg–Marquardt algorithm with double precision. In all experimental cases the equation for the distribution of the data was satisfied.

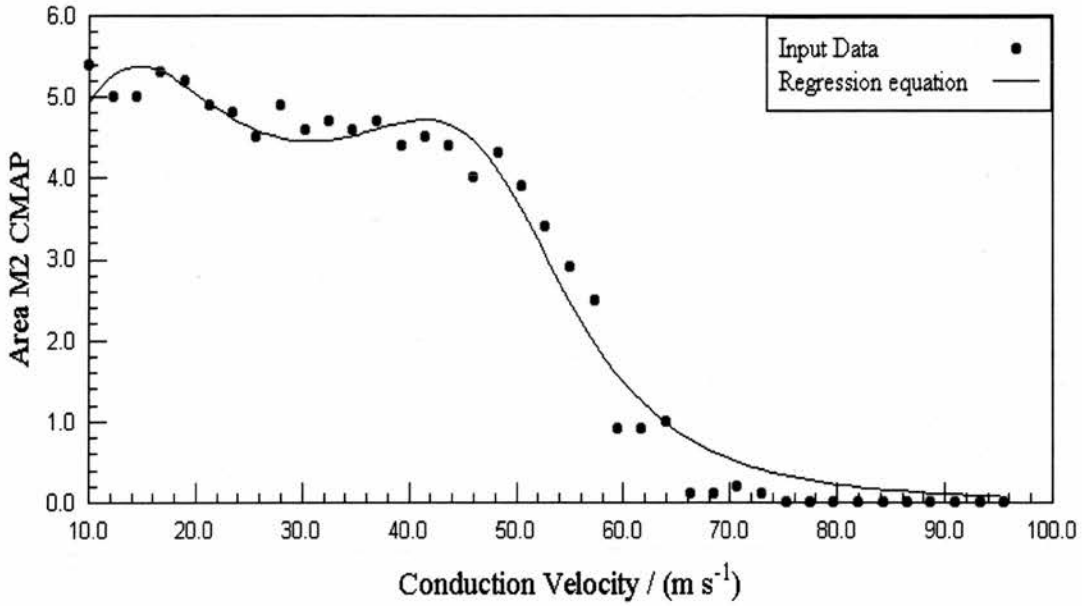


Figure 1

An example of experimentally determined data after 'curve-fitting by Datafit. The blue dots are the individual values of M2 for each conduction velocity (*aCV*) and the black line is the computed best-fitting function.

The numerical values for the constants in the computed polynomial equation {Equation 4} of the best-fitting line were returned to the Excel spreadsheet where the equation was differentiated. This allowed the gradient for each point along the line to be calculated. The gradient of the line at any point corresponded to the change in the proportion of active fibres at a corresponding conduction velocity. These values were plotted to give a graphical representation of the distribution of conduction velocities for the nerve under investigation. Figure 2 is an example of such a graph resulting from an experiment to distinguish neurapraxia and axonotmesis from normal conduction in the sheep median nerve. Since CMAPs have been measured throughout, this is a purely motor CV_{Dist}. The two peaks seen in the normal nerve probably correspond to populations of α-motoneurons and γ-fusimotorneurons. In both types of injury there has been a shift to the left of the faster fibres in keeping with the

expected notion that large fibres never recover fully after injury (Cragg & Thomas 1964; Gattuso, Glasby, & Gschmeissner 1988; Glasby, Gattuso, & Huang 1988). In the more severe injury of axonotmesis there are many fewer fibres conducting impulses.

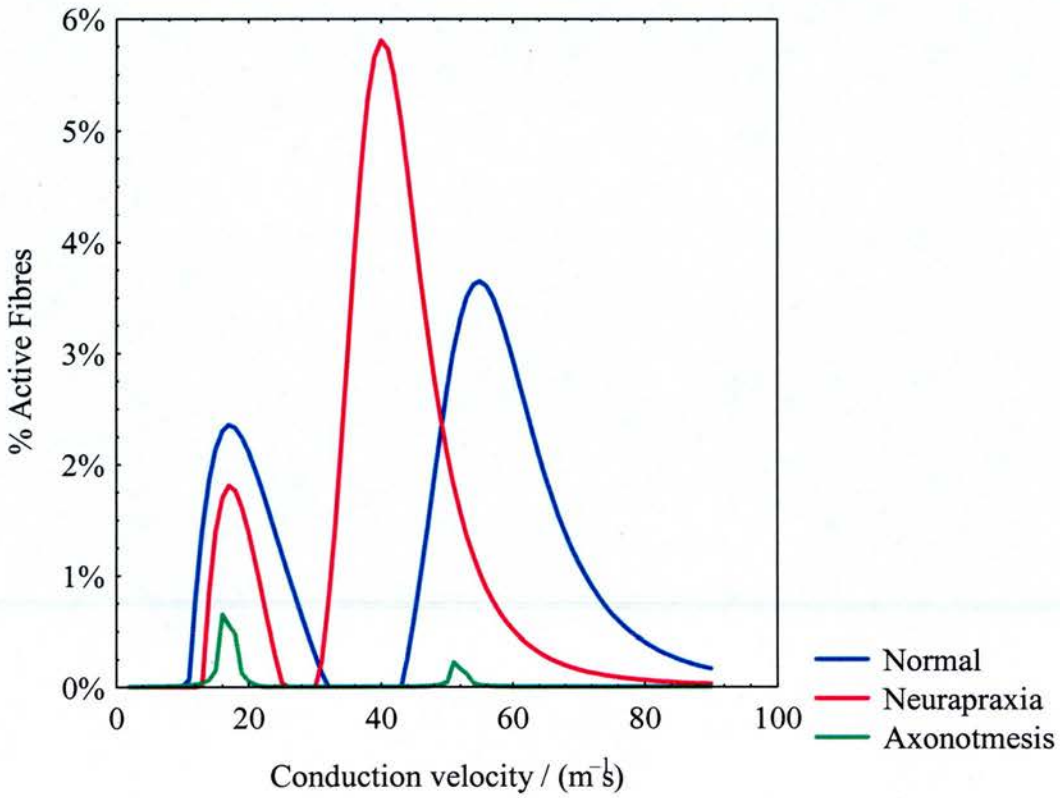


Figure 2

CV_{Dist} measurement in a sheep after the creation of lesions of neurapraxia and axonotmesis created as described in Chapter 2 and compared with CV_{Dist} for normal sheep median nerve. After both types of injury there has been a leftward shift and in axonotmesis a considerable decline in the number of active fibres.

TESTS CONFINED TO THE LABORATORY

Two groups of tests may be identified. The first consists of isometric tension measurements directed at the target muscles of reinnervation. The second group comprises morphological tests requiring sampling of the repaired nerve. Also sometimes included in this group may be electrophysiological assessment of the entire motor pathway when the brain or spinal cord has been exposed for stimulation. Early morphological tests involved merely looking at wax-embedded specimens of about 7μm thickness. Advancing techniques of tissue preparation derived from electron microscopy have enabled resin-embedded sections to be cut at 1μm for light microscopy with greatly increased resolution of detail. Coincidentally the development of computerized morphometry has made possible the accurate estimation of morphological indices of nerve regeneration and although not directly measuring function, these have become a 'gold-standard' in the assessment of nerve injury. They are:

1. Isometric tests of muscle function
    - Twitch properties
    - Tetanic properties
    - Muscle mass
2. Morphometric analysis
    - Fibre diameter
    - Axon diameter
    - Myelin sheath thickness
    - G-ratio (axon diameter ÷ fibre diameter)
    - Internodal length

In most cases the best information is gained from an examination of the distribution of these variables rather than from their mean values. A regression graph of internodal length against fibre diameter has been shown to be particularly sensitive as a measure of recovery and of the effects of injury and method of repair (Gattuso 1988; Gattuso, Glasby, & Gschmeissner 1988; Kelleher 2004).

Estimation of recovery of muscle function by measurement of isometric tension

These studies are based upon classical Newtonian mechanics, the studies upon the production of heat by working muscle carried out by A.V.Hill and colleagues and the electrophysiological studies on nerve and muscle by the first Lord Adrian. An early monopoly on this work is thereby claimed by Trinity College Cambridge. Muscle physiology is useful in the study of nerve injury and regeneration because it provides information about the function of the motor end-organ. When a propagated nerve impulse reaches the neuromuscular junction (NMJ) it results in the release of acetylcholine (ACh) into the synaptic cleft. ACh, in binding to receptors on the subsynaptic membrane causes the membrane potential to approach zero by virtue of the increased permeability to sodium which in turn initiates a muscle action potential which is conducted to the myofibrils by a system of T-tubules. The muscle action potentials cause a release of Ca^{2+} ions from the sarcoplasmic reticulum which leads to muscle contraction. This process is termed excitation-contraction coupling.

When Ca^{2+} ions are so released from the sarcoplasmic reticulum they bind to troponin C and disinhibit the troponin-tropomysin complex to make the contractile proteins actin and myosin available to do their work and allow contraction to proceed. Energy for this process is supplied by adenosine triphosphate (ATP) binding to the heavy

meromyosin fragment. ATP is supplied from three sources; creatine phosphate, the Embden–Meyerhof pathway and as a by-product of the Krebs' cycle. Mammalian skeletal muscle contains three types of histochemically distinguishable fibre; red, slow-twitch fibres, white, fast-twitch fibres and red, fast-twitch fibres (Close 1964; 1965a; 1965b; 1967; 1969; 1972a). Red, slow-twitch fibres contain high levels of myoglobin and large numbers of mitochondria to facilitate oxidative phosphorylation. They are resistant to fatigue and predominate in tonic, postural muscles. White, fast-twitch fibres have a well-developed glycolytic enzyme-system and show high myosin-ATPase activity. They fatigue rapidly and are predominantly found in phasic muscles. Red, fast-twitch fibres are intermediate between red, slow fibres and white, fast fibres.

Muscle fibres of any motor unit may interdigitate anatomically with other motor units (Burke 1980). The fibres are spread out through the muscle in microbundles of three to fifteen fibres and muscle fibres of a single motor unit are the same type (Kugelberg, Edstrom, & Abbruzzese 1970).

When a muscle is denervated there is flaccidity and paralysis, the blood supply to muscle is decreased owing to loss muscle pumps and by forty-eight hours after denervation the resting membrane potential has decreased and become more sensitive to ACh. This results in spontaneous fibrillation which may persist for as long as the contractile elements within the muscle survive. Macroscopically, denervated muscle becomes paler and decreases in both size and weight which is most marked in the first two months after denervation then stabilizes (Sunderland 1978).

Reinnervation of muscle occurs when pioneering axons reach motor end-plates and form new NMJs. However before the return of precision and power of motor function maturation must also occur. Good return of function has been observed in humans

after periods of denervation of a year (Sunderland 1978; Sunderland 1991). Factors associated with a poor outcome in the return of motor function include a reduction in the number of effective axons, mismatching at the site of repair and a subsequent abnormal pattern of reinnervation, abnormalities of central activation and irreversible changes in the muscle fibres (Sunderland 1978; Sunderland 1991).

Properties of isometric twitches and tetani

Measurement of muscle contraction may be carried out isotonically or isometrically. In isotonic contraction muscle performs work against a fixed load and the characteristics of the contraction depend on the size of the load and its inertia. In isometric contraction the length of the muscle is held constant so that the force measured is purely that generated by the contractile mechanism. Isometric tension measurement is thus more appropriate in physiological studies.

Twitch time

Isometric twitch time is postulated to be increased after nerve injury. The rationale is that after nerve transection, muscle is reinnervated by thinner, slower axons and it is known that that both speed of muscle contraction and muscle-fibre type are influenced by the type of innervation. The isometric twitch time includes both the time for muscle to contract and also the time for muscle to relax. However the relaxation phase may be prolonged to the point of becoming asymptotic since the viscous properties of the muscle fibres are predominant in relaxation. In the present experiments measurements were made of the time to peak twitch tension (*time to peak twitch*) and of the time for the muscle to relax to half the peak twitch tension (*time to half relaxation*). The time to half-relaxation of the muscle was a point which could be

easily standardized and occurred before the relaxation curve became asymptotically prolonged.

Twitch tension and tetanic tension

Muscle contains a contractile i.e. viscous component and a series-elastic component. In engineering terms it is a visco-elastic 'dashpot' in parallel with an elastic (spring) element and both of these are in series with a further elastic element. The spring (elastic) elements obey Hooke's Law[17]. The pure elastic component consists of non-contractile structures such as connective tissue, sarcolemma, blood vessels and nerves. These structures stretch during muscle elongation. There is no tension in an unstimulated muscle of resting length. However, as muscle is stretched the amount of tension increases owing to the effect of the elastic component. This is termed the resting or passive tension. The tension which develops as a muscle contracts is termed the active tension and is brought about by the conformational changes which take place in the ATP-dependent contractile proteins.

In order to test the maximal ability of a muscle to generate isometric tension, it must be stimulated tetanically. This can be achieved with a stimulating frequency which is greater than the fusion-frequency of muscle contraction. The latter varies with fast and slow muscles but a stimulating frequency of 100Hz may be relied upon always to cause tetanization of the muscle.

The tension generated in a muscle held isometrically depends upon its initial length. The maximum isometric tetanic tension is generated when a muscle is stretched to 120% of its resting length, prior to stimulation (Close 1972b). According to the sliding filament theory maximum tetanic tension is obtained when there is maximum

[17] Quite beautiful for its concision — *ut tensio, sic vis.*

overlap of thick and thin filaments. If the muscle length is too short the thick filaments are limited because they oppose each other at the Z-bands. Also, the process of activation of the muscle is thought to be less efficient at shorter lengths. Conversely if the muscle is stretched so that the length of the sarcomere exceeds the combined length of the thick and thin filaments (*i.e.* there is no overlap), there is a linear decline in tetanic tension (Gordon, Huxley, & Julian 1966a; Gordon, Huxley, & Julian 1966b). The ratio of peak isometric twitch tension to isometric tetanic tension, in adult mammals at 37°C, is 1: 3.5 (Close 1972a; Close 1972b).

The optimal muscle length for the generation of reproducible isometric twitches is 5% to 10% greater than that for isometric tetanic tension i.e. 125% of resting length. The optimal length of a sarcomere to generate the maximum twitch tension is 2μm (Gordon, Huxley, & Julian 1966a; Gordon, Huxley, & Julian 1966b).

Isometric twitch tension is a function of the number of active motor units and the repetitive frequency at which these units contract (Lippold 1952). This may be related to the [Ca^{2+}] ions released. Desmedt and Hainaut showed that repetitive stimulation of muscle resulted in an increase of twitch tension to 136% of that initially obtained (Desmedt & Hainaut 1968). The speed of muscle contraction and relaxation was decreased coincidentally. This may be due to a greater turnover of Ca^{2+} ions.

Burke showed that fast motor units generate more tension than slow motor units (Burke 1967). Luff *et al*, also in the cat, found that fast-fatiguable motor units generated higher tensions than fast-non-fatiguable units which in turn generated more tension than slow units (Luff, Hatcher, & Torkko 1988).

When an isometric twitch was produced by the delivery of a single stimulus to a muscle, Hartree and Hill showed that if H = heat, T = isometric twitch tension and l = muscle length, H/Tl had a constant value. They proposed that isometric twitch tension

was the best measure of the mechanical response of a muscle (Hartree & Hill 1921). However, an isometric twitch contraction does not allow adequate time for the contractile mechanism to develop maximum tension. Furthermore, the tension recorded at the muscle tendon is diminished by the passive elastic component which is still stretching (Desmedt & Hainaut 1968).

Individual twitch contractions can summate to increase the overall intensity of muscle contraction. Summation can be achieved either by the recruitment of motor units or by increasing the frequency of activation. Ultimately a (maximum) fusion-frequency is reached and the muscle becomes tetanized. In multiple fibre summation low levels of stimulation cause activation of the smallest motor units and as the stimulation strength increases, progressively larger motor units are recruited. This is known as the size principle.

Two further measurements, relating to muscle tension, were used in the present experiments. These were time tension integral ($TT\int$) and time tension index *(TTI)*. $TT\int$ is the total force generated by the muscle (in the EPNRL to the point of half-relaxation of the twitch contraction or half-fatigue of the tetanic contraction). It is found by measuring the area under the time-tension curve for the twitch or tetanus.

$TT\int$ is equivalent to the momentum (mass× velocity) produced in a heavy suspended mass, by muscle contraction (Hartree & Hill 1921). It therefore reflects the ability of a muscle to move heavy loads. Hartree and Hill found a linear relationship between the $TT\int$ and the amount of heat produced by the muscle. *TTI* represents the average force generated by the muscle, to the point of half relaxation. It was calculated by dividing $TT\int$ by the time to half relaxation.

Experimental measurement of isometric twitch and tetanic tension

The operating theatre set-up and anaesthesia were as described in Chapter 2. neuromuscular blockade was not used. This phase of the experiment usually followed on from that in which CV_{max}, CV_{Dist} etc were measured. The median nerve in the arm had therefore been exposed.

It was essential in measuring isometric tension that the force was measured in a straight line and that the limb and tension transducer were anchored immovably to the same base. This base, in the present experiments was a steel bar of 2.5cm square cross-section which was fixed to the side bar of the operating table. It was thus very rigid but accommodated any changes in height or orientation of the table. At its proximal end was a rigid fixation-frame into which the proximal part of the forelimb of the sheep could be rigidly fixed by means of a self-tapping screw passed through the olecranon process. The olecranon was exposed and cleared of soft tissue by means of a rongeur. Using an air-powered drill passed through a guide to protect it from soft tissue, a hole was drilled through the olecranon and this hole was tapped with a hand tap also passed through the guide. These instruments are those provided in the A.O. Small-fragment Sets, (Synthes, UK). A 6cm self-tapping lag screw was then passed through the pre-tapped hole and positioned so that approximately equal lengths of the screw protruded on either side of the olecranon. The screw was then fixed into the fixation-frame for the olecranon.

The tension transducer (Grass Instruments, U.S.A.) was fixed distally to the steel bar ready for attachment of the tendon of the muscle under test. It was carried on a sliding plate which ran in grooves on each side of the bar and could be locked into position by means of an Allen key. In this way the distance between olecranon and transducer and hence the initial length of the muscle could be varied.

The distal part of flexor carpi radialis (FCR) muscle was now exposed adjacent to the bigger flexor carpi ulnaris (FCU) muscle and on top of flexor digitorum superficialis and flexor digitorum profundus. FCR and FCU were enclosed in a common fascial sheath which was opened. The proximal portion of FCR was left untouched so as not to compromise its blood supply and innervation. The tendon of FCR was dissected free to its insertion, and divided as distally as possible.

The resting length of the muscle was measured from fixed datums on the steel bar. A length of heavy linen thread was tied around the tendon and attached to the tension transducer. The initial length was set to 125% of the resting length and appropriate datums were marked so that the muscle could be returned to this resting length for further measurements.

Stimulation of the median nerve was carried out as described previously using the Medelec machine. This could be programmed to deliver single square wave stimuli or trains of square waves at a predetermined frequency. Tetanization was achieved with stimulation frequencies of 100Hz. Since the Medelec machine could not receive an input from the force transducer it was used to trigger an 'oscilloscope' into whose Y input the output of the tension transducer was directed. The term 'oscilloscope' is placed in inverted commas as it was not a true cathode ray or digital oscilloscope but an analogue-to-digital converter (Picoscope ADC 216; Pico Technology Ltd The Mill House, Cambridge Street St.Neots, Cambs PE19 1QB U.K.). The output from this ADC passed through the parallel port of a PC and was displayed using the appropriate Pico software. This had all the features of a true oscilloscope including the ability to make measurements by means of cursors. In this way all linear measurements could be made directly. In order to measure areas the curve could be exported as a text file into Excel and Datafit (see above) and integrated in the latter.

Calibration of the tension transducer was carried out beforehand by suspending weights on the transducer beam and recording the d.c. deflection on the oscilloscope. A calibration graph was thus constructed.

Twitch tension measurement

The following measurements were recorded:

(i) ***Amplitude of peak twitch*** (N). This represented the maximum force generated by the twitch contraction. This was the distance along the y-axis from the take-off point of the twitch-tension curve to the point of maximum amplitude.

(ii) ***Time to peak twitch*** (ms). This was the time from the onset of muscle contraction to point of maximum contraction. On the twitch-tension curve this was the distance along the x-axis from the take-off point to the point of maximum amplitude.

(iii) ***Time to half relaxation*** (ms). This was the time from the point of maximum muscle contraction to the point where the muscle had half-relaxed. This point was found *post hoc* by means of the cursors on the Picoscope.

(iv) ***Time tension integral*** —$TT\int$ (mNs). This represented the total force generated by the twitch contraction to the point of half relaxation of the muscle. This was the area under the twitch tension curve from the take off point to the point on the down slope of the curve which was half the amplitude of the maximum amplitude of the curve.

(v) ***Time tension index*** — *TTI* (N). This was calculated by dividing the time tension integral by the time to half relaxation.

Tetanic tension measurement

The following measurements were recorded:

Maximum tetanic tension (N).This was the maximum tetanic force generated by the muscle. This was measured on the y-axis from the baseline to the point of maximum amplitude of the tetanic-tension trace.

Time to half fatigue (s). This was the time from the start of muscle contraction to that point when the muscle had fatigued to half of its maximum force. It was measured on the y-axis from the take-off point to the point where the trace was half of the maximum amplitude.

Time tension integral (Ns). This was the area under the tetanic tension curve from the take off point to the point of half fatigue.

Time tension index (N). This was calculated as above.

Muscle mass

The change in muscle mass after reinnervation is a good index of the level of recovery of contractile function. Ten to twenty five percent of normal muscle consists of connective tissue which may be expected to remain roughly constant whilst the mass of contractile tissue decreases and is ultimately replaced by scar tissue. Weiss and Eds transected the sciatic nerves of rats and found that the weight of the leg muscles fell to 22% of normal (Weiss & Edds 1946). They suggested that the decrease in muscle mass associated with denervation was a result of the loss of contractile tissue. However, denervation experiments in the somewhat unusual (characteristically antipodean) model of the opposum demonstrated that skeletal muscle retained its histological characteristics 485 days after loss of its nerve supply (Sunderland 1978). Bowden and Gutmann found normal fibres in human muscle three years after denervation (Bowden & Gutmann 1944). Gutmann performed denervation experiments on rabbits and found that by twenty months muscle had degenerated but retained the ability to form new end-plates and to regenerate on reinnervation

(Gutmann 1948). As muscle degeneration progresses, fibroblasts proliferate and there is a relative increase in the amount of connective tissue with thickening of the perimysium and endomysium. Muscle fibres may be replaced with fibrous tissue which can result in shortening of muscle and the formation of contractures. The changes in weight are easily quantifiable and thus provide a useful albeit invasive index of progress.

Morphometric evaluation of the tissue specimens

It has been traditional to use anatomical and more latterly morphometric indices as indicators of the efficacy of nerve regeneration after injury and repair. In particular great emphasis was paid, in the past, to counting the number of regenerated nerve fibres. This is a dangerous strategy: several authors have fallen into the trap of sampling axons from a very small portion of the nerve and then multiplying by the total cross-sectional area. This takes no account of the fact that a large proportion of normal nerve is not composed of neural tissue. Some embarrassing overestimations have emerged and, more worryingly, found their way into respected journals (Fawcett & Keynes 1990). Moreover the logic is flawed since it is known that the initial profuse sprouting of the pioneering axons becomes checked as they fail to make peripheral connections. In theory, therefore, a count of axon numbers in regeneration can only be valid if one is certain that the regenerative process has reached its end-point and this is a virtually impossible point to define and recognize.

A more recent and much better strategy has been to examine the morphological characteristics of the regenerated fibres and to compare them with normal values. The advent of computerized morphometric systems has greatly facilitated this. Four quantities have become established as useful indices of recovery. Two of these, axon diameter and fibre diameter are measured and two, myelin sheath thickness and g-

ratio are derived by calculation from the two measured variables. The virtue of g-ratio (axon-diameter ÷ fibre-diameter) as a measurement is that it is an index of fibre maturity for a given axon diameter. It is well known that after injury recovery is never complete to normal levels. Nevertheless for a given level of recovery of axon diameter, myelination may be normal for that diameter or not. The measurement of g-ratio allows this quantity to be evaluated.

After injury, as the nerve matures, fibre diameter and myelin sheath thickness increase but never regain normal levels. Morphological measurements are therefore indicators of both nerve regeneration and maturation. Of course, all of these measurements while undoubtedly very convincing because of their absolute nature are of no use clinically because they are obtained only after the destruction of the nerve. However fibre diameter correlates well with the clinically easy-to-measure variable CV_{max} and this correlation is most reassuring. Several experimental studies have shown that for 'geometrically similar' myelinated fibres, CV_{max} is almost proportional to fibre diameter (Waxman 1980). Hursh demonstrated in a variety of kitten and adult cat nerves that the maximum conduction velocity of a nerve could be predicted by multiplying the diameter of the largest fibre (μm) by six (Hursh 1939). However Boyd found that the ratio between fibre diameter and conduction velocity was lower for γ fibres than for α fibres. The coefficients he derived for conversion of fibre diameter (μm) to conduction velocity (m s^{-1}) were 5.6 for α fibres, 4.4 for fast γ fibres and 4.5 for slow γ fibres (Boyd 1964). Computer simulation studies have also confirmed the proportional relationship between fibre diameter and conduction velocity (Goldman & Albus 1968; Rushton 1951) .

Conduction velocity is also affected by myelin sheath thickness (Smith & Koles 1970). Conduction velocity appears to be optimized at a g-ratio between 0.6 and 0.7

(Goldman & Albus 1968; Moore, Joyner, Brill, Waxman, & Najar-Joa 1978; Rushton 1951; Smith & Koles 1970). Brill et al found that myelin sheath capacitance as well as thickness affected conduction velocity (Brill, Waxman, Moore, & Joyner 1977).

The effect of nerve injury and repair is to reduce the value of CV_{max} and this never recovers to normal levels. Berry *et al* transected the tibial, peroneal and saphenous nerves of cats and performed immediate epineurial suture repair. In these experiments CV_{max} was reduced by more than 20% of the normal value (Berry, Grundfest, & Hinsey 1944). These authors also showed a correlation with a reduction of 20% in the diameter of the regenerated fibres.

Cragg and Thomas crushed the peroneal nerve in adult rabbits. In the regenerated nerves they observed a reduction in CV_{max} of 25% compared with normal controls (Cragg & Thomas 1964). In the distal nerve segment they measured a small reduction in fibre diameter, a normal g-ratio and a reduction in internodal length of more than 50%. These workers did not think that the measured reduction in CV_{max} could be completely accounted for by the small decrease in fibre diameter but neither did they attribute it entirely to the decrease in internodal length. The measurement of internodal length is possible though tedious as it requires the painstaking teasing of osmicated nerve fibres in glycerol (Gattuso 1988; Gattuso, Glasby, & Gschmeissner 1988; Kelleher 2004). Gattuso (*op cit*) demonstrated most convincingly the value of examining a regression plot of nerve fiber diameter against internodal length. This is probably the most sensitive (and most difficult and time-consuming) morphometric test available for studies on peripheral nerve injury and repair.

The preparation and use of 1μm resin-embedded nerve sections for light microscopy and morphometry.

The resolution obtained when wax embedded sections of nerve (usually at about 7μm thickness) are viewed with the compound microscope is too poor for accurate morphometry. This is overcome by the expedient of using a modification of the technique used for preparing specimens for electron microscopy. Embedding is in Araldite resin and the sections are cut on an ultramicrotome but at 1μm. When viewed by means of the light microscope these give excellent resolution and are ideal for morphometric studies.

Fixation

Specimens of 2cm lengths of nerve were harvested, distal to the site of injury in all experiments and placed in a 4% cacodylate-buffered gluteraldehyde solution for one hour at room temperature. They were subsequently trimmed with a razor blade under a low-power microscope in order to produce 1mm slices with, as far as possible, flat surfaces. They were replaced in fresh 4% cacodylate-gluteraldehyde solution and left overnight at room temperature. The next morning the sections were washed for twenty minutes three times in sucrose buffer then immersed in 1% cacodylate-buffered osmium tetroxide for three hours to allow impregnation of the myelin with reduced metallic osmium. The sections were next dehydrated in three washes of 10% alcohol times for thirty minutes each wash in then again three times each for thirty minutes in 100% alcohol. This was followed by one thirty minute wash in propylene oxide.

Embedding

The sections were removed from the propylene oxide, placed in plastic moulds which were filled with liquid Araldite. These were left overnight at room temperature to

allow penetration of the Araldite into the nerve. The next morning excess Araldite was removed and the specimens were transferred to smaller moulds which were filled with fresh Araldite. One or two sections were placed in each mould and orientated with a cut edge parallel to one of the sides of the mould. The moulds were placed in an oven, maintained at a temperature of 60°C, and left for at least forty-eight hours to allow polymerization of the Araldite.

Cutting and Staining

After the Araldite was set the specimens were removed from the moulds and trimmed. Each was then mounted on a half-centimetre length of 0.5cm dowel rod using molten sealing-wax. The dowel could then be held in the chuck of the ultramicrotome where they were cut at 1μm thickness with a freshly prepared glass knife. Wrinkles in the sections were minimized by exposing them to chloroform. The sections floated onto glass microscope slides were gently dried on a hotplate and stained with 1% toluidine blue in 1% sodium tetraborate. The toluidine blue stain highlighted all tissues in addition to the myelin sheaths impregnated with osmium.

Digital imaging

Each section was viewed using a binocular microscope. An array of images of each section were digitized using an oil immersion objective (×100). To the observer, using a ×10 eyepiece this gave a magnification of ×1000. A beam-splitter allowed the microscope to be connected to a digital camera which enabled each image to be displayed on a monitor screen and stored as a TIFF file. The images were later recovered and analysed using an image analysis program (Analytical Imaging Systems (AIS), version 3.0, Imaging Research Inc, Canada).

Morphometric analysis

Axon diameter and fibre diameters were measured using the AIS program. Measurements were made by placement of a cursor across the shortest diameter of the fibre or axon. The shortest diameter was used as it exhibits least variation of the section has not been cut precisely at right angles. Two hundred measurements were made for each nerve as this has previously been shown to be representative of the whole nerve (Mayhew 1990; Mayhew 1988). Results were exported into Excel spreadsheets where myelin thickness and g-ratio were calculated. Myelin sheath thickness was calculated using the formula:

$$\frac{Fd - Ad}{2}$$

Where:

Fd = fibre diameter (μm)

Ad = axon diameter (μm)

and g-ratio is defined as:

$$\frac{Ad}{Fd}$$

Statistical treatment of experimental data

The method of statistical analysis used throughout the present experiments is that which has been developed in the Peripheral Nerve Research Laboratory, Department of Clinical Neurosciences, University of Edinburgh (EPNRL) for the appraisal of models of nerve injury and their surgical repair.

Raw data were first organized into a spreadsheet using the computer programme 'Microsoft Excel'. The extreme left hand column contained a list of identification numbers of the individual data points/experimental subjects *etc.* In a column

immediately to its right the independent variable '*Experiment*' was placed as a list in which the subgroups *i.e.* specific experimental procedures were designated. In subsequent columns to the right of this, each dependent variable was recorded. When the Excel spreadsheet was completed with all of the morphological and physiological results it was imported into the statistics computer programme ('Statistica version 6.0' — Statsoft Inc, 2300 East 14th Street, Tulsa, O.K. 71404, U.S.A.). This programme is an extensive package for statistical analysis and graph plotting.

The statistical appreciation of how group sizes are determined is summarized in the accompanying flowchart of the statistical algorithm (Appendix 2). First, it was necessary to identify and reject outliers within the raw data. This was accomplished by plotting *half-normal probability plots* in which the selected variable was plotted in a scatterplot against the values calculated as those 'expected from the normal distribution with the same mean and variance' for each column of data. The *half-normal probability plot* was constructed in the same way as the standard *normal probability plot* (see below), except that only the positive half of the normal curve was considered. Consequently, only positive normal values were plotted on the *Y-axis*. On each plot it was possible to draw an ellipse representing the 95% confidence intervals for the distribution. Points lying outside this ellipse were considered to be outliers and rejected from the study. The resulting spreadsheet was termed 'weeded data'.

After rejecting the outliers, the columns of data were used to determine the residuals of each variable and the residuals were then re-plotted as *normal probability plots* in order to determine whether or not the data fitted a *normal distribution*. It is better to use residuals rather than the raw data themselves as often the distribution of data points may not be normal when the distribution of the residuals is, and it is the latter that matters. With this technique there was less need to use relatively insensitive non-

parametric tests. The standard *normal probability plot* was constructed as follows. First, the values were rank ordered. From these ranks, *Z values* (i.e., standardized values of the normal distribution with the same mean and variance as the data) were computed based on the assumption that the data came from a normal distribution. These *Z values* were plotted on the y-axis in the plot and if the observed values (plotted on the x-axis) were normally distributed, then all values should have fallen onto a straight line in the plot. If the values were not normally distributed, they would have deviated from the line. The fit of the computed line to the scatter of the raw data was tested by the programme using the *Shapiro–Wilk W test*. If the *W statistic,* so produced was significant, then the hypothesis that the respective distribution was normal had to be rejected. The *Shapiro-Wilk W test* is the preferred test of normality because of its good power properties as compared to a wide range of alternative tests (Shapiro, Chen, & Wilk 1968). At this point, for ease of recognition, each of the columns in the spreadsheet of dependent variable data that were not normally distributed were converted to a red fount for easy identification.

The next two stages in statistical testing were directed at identifying the presence of differences (variants of the *F test*) and identifying where these differences lay (variants of *Student's t test*). Different algorithms had to be adopted for normally and non-parametrically distributed data.

For normally distributed data, the *F test* was applied in the form of one way ANOVA (analysis of variance). The independent variable with its subgroups was the 'grouping' or 'factor' in the ANOVA calculation and the dependent variables were the columns of weeded data. The ANOVA test identifies differences due to 'between-groups variation' whilst neutralizing differences due to 'within-groups variation' From the ANOVA test 'p' values for statistical differences were identified. In order to

find where these differences lay it was necessary to perform *post hoc* tests which tested the null hypothesis by comparing the means of these independent groups of data. The paradigm for this is *Student's t test* which compares the means of two independent samples. This test asks the question: 'What is the probability that the two samples (represented by their means and variances) were drawn from the same population?'. It is important to disabuse oneself of the erroneous notion that the test tests for 'significant differences': this is an all too common misconception[18]. The Student's t-test, is not however appropriate where ANOVA discloses multiple differences (or not as may be the case) and this is likely in experiments such as those encountered here. If repeated t tests were to be performed on a single cohort of data, there would be an increased likelihood of Type I error in the analysis. Type I error is the acquisition of 'false positives'. In order to reduce this occurrence when, within the independent variable there are more than 2 subgroups (represented in Statistica by 'codes'), the more conservative *Scheffé test* is used in preference to the t-test. It is a variant of the *t test* specifically for use in the situation of analysis of variance where multiple groups are being considered.

On the rare occasions when data were found not to be parametrically (normally) distributed, statistical analysis based upon the ranking of data had to be used. There is a variety of tests equivalent to the more sensitive parametric tests used above. The *Kruskal–Wallis test* is a non-parametric alternative to one-way (between-groups) ANOVA. It was used to compare three or more samples, and it tests the null hypothesis that the different samples in the comparison were drawn from the same distribution or from distributions with the same median. Thus, the interpretation of the *Kruskal–*

[18] With a distressingly high incidence in Surgical Journals.

Wallis test is similar to that of the parametric one-way ANOVA, except that it is based on ranks rather than means.

In the case of non-parametrically distributed data for testing where the differences lay, two different tests were employed. For small numbers of data points (N=5 to N=30) the Mann–Whitney U test was used. The Mann–Whitney U test assumes that the variable under consideration has been measured on at least an ordinal (rank order) scale. The interpretation of the test is essentially identical to the interpretation of the result of a t-test for independent samples, except that the *U* test is computed from rank sums rather than means. The *U* test is the most powerful (i.e. sensitive) nonparametric alternative to the t-test for independent samples; in fact, in some instances it may offer even greater power to reject the null hypothesis than the t-test.

Where there was a large number of distributed data (*e.g.* N=200 e.g. obtained for morphometric tests) these were analysed by a test which more readily took into account the shape of the distribution i.e. the Kolmogorov–Smirnov test. This assesses the hypothesis that two samples were drawn from the same population but unlike the parametric t-test for independent samples or the Mann-Whitney U test, which test for differences in the location of two samples (differences in means, differences in average ranks, respectively), the Kolmogorov–Smirnov test is also sensitive to differences in the general shapes of the distributions in the two samples (i.e., to differences in dispersion, skewness, kurtosis etc.).

Statistical Power

In any study where statistics have been used it is important to consider the statistical power of one's tests. Statistical power is precisely defined as: 'The probability of rejecting a false statistical null hypothesis'. An error of this type is

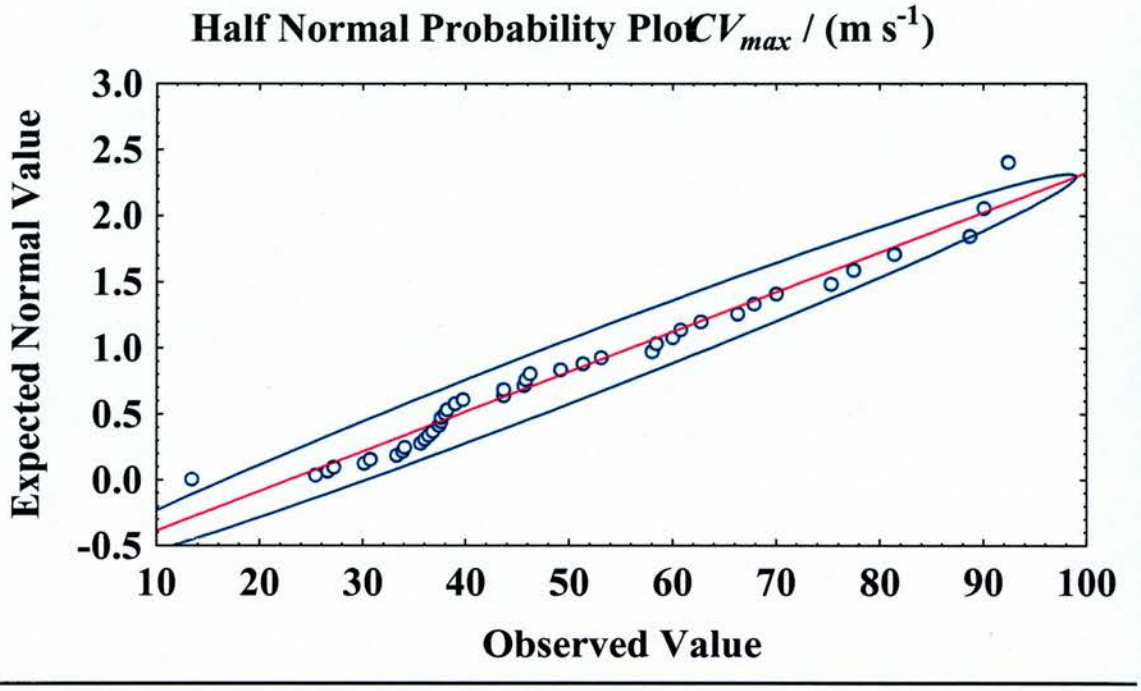


Figure3

An example of a half normal probability plot for facial nerve for the variable CV_{max}. Two points lie outside the 95% ellipse and are therefore regarded as outliers.

termed a Type II error and is the failure to reject a false null hypothesis, effectively a false negative. The probability of detecting a Type II error is defined as:

$$p\ (\text{Type II}) = \beta$$

The power of a test is therefore 1-β: ideally it should be 1.0 but an experimental value of 0.9 is considered excellent. It is thus important in predicting the useful size of samples before undertaking experiments and, at the end, in assessing the accuracy of the analysis that was used. 'Statistica' contains a comprehensive '*Power Analysis*' module which allows these tests to be carried out. Using this it was possible to test the power of each of the tests which had been performed for each of the dependent variables. It was then possible to compare these results retrospectively with the computations for sample size which had been made at the start of the project.

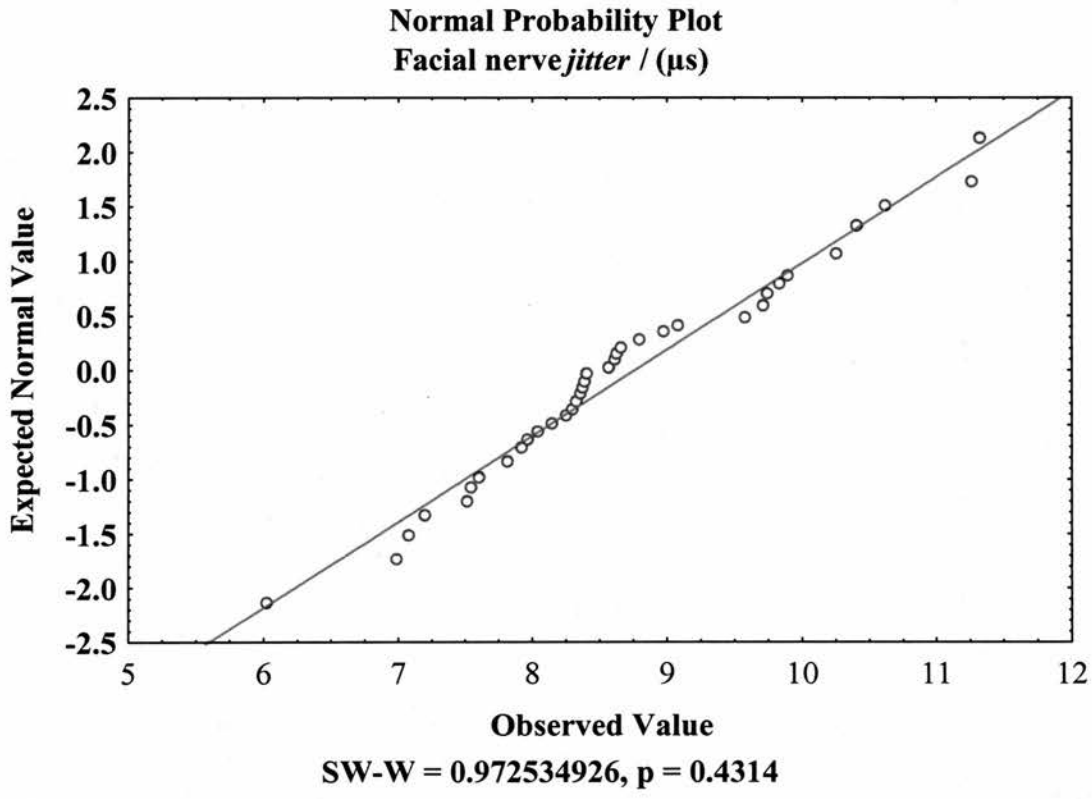


Figure 4

A normal probability plot for *jitter* in the sheep facial nerve. As the probability of the two samples having been drawn from the same population is greater than 5% ($p>0.05$) the data are normally distributed.

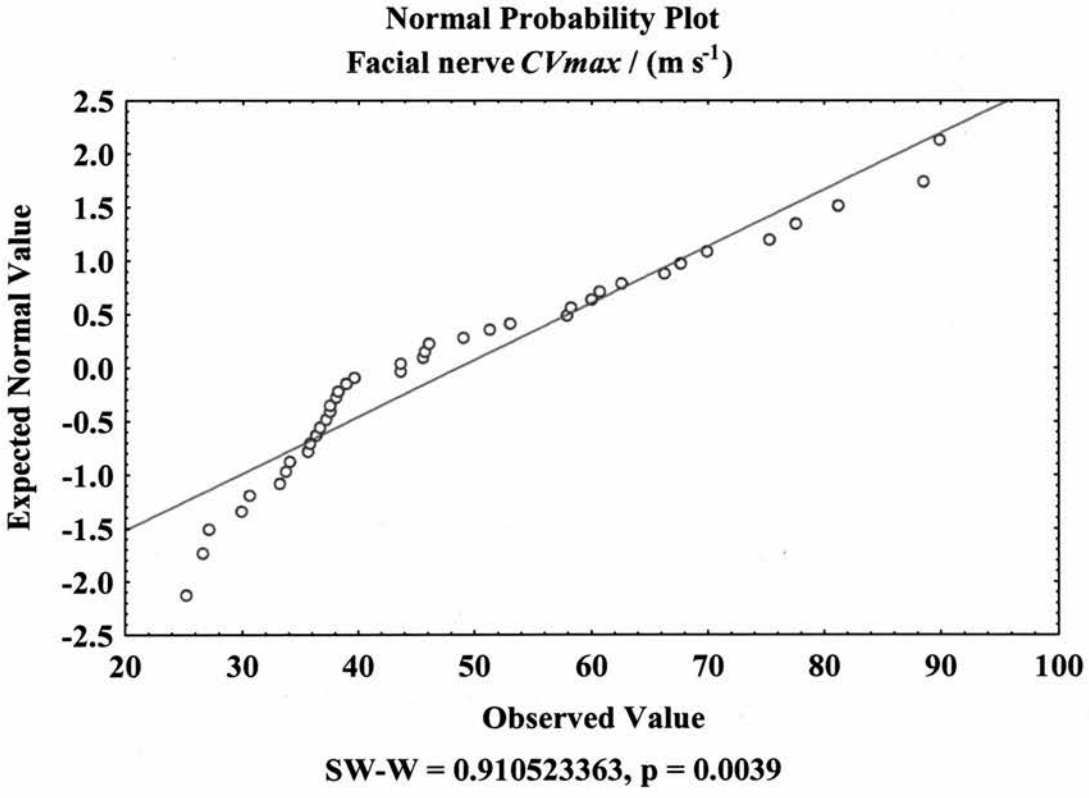


Figure 5

A normal probability plot for CV_{max} in the sheep facial nerve. As the probability of the two samples having been drawn from the same population is less than 5% ($p<0.05$) the data are not normally distributed.

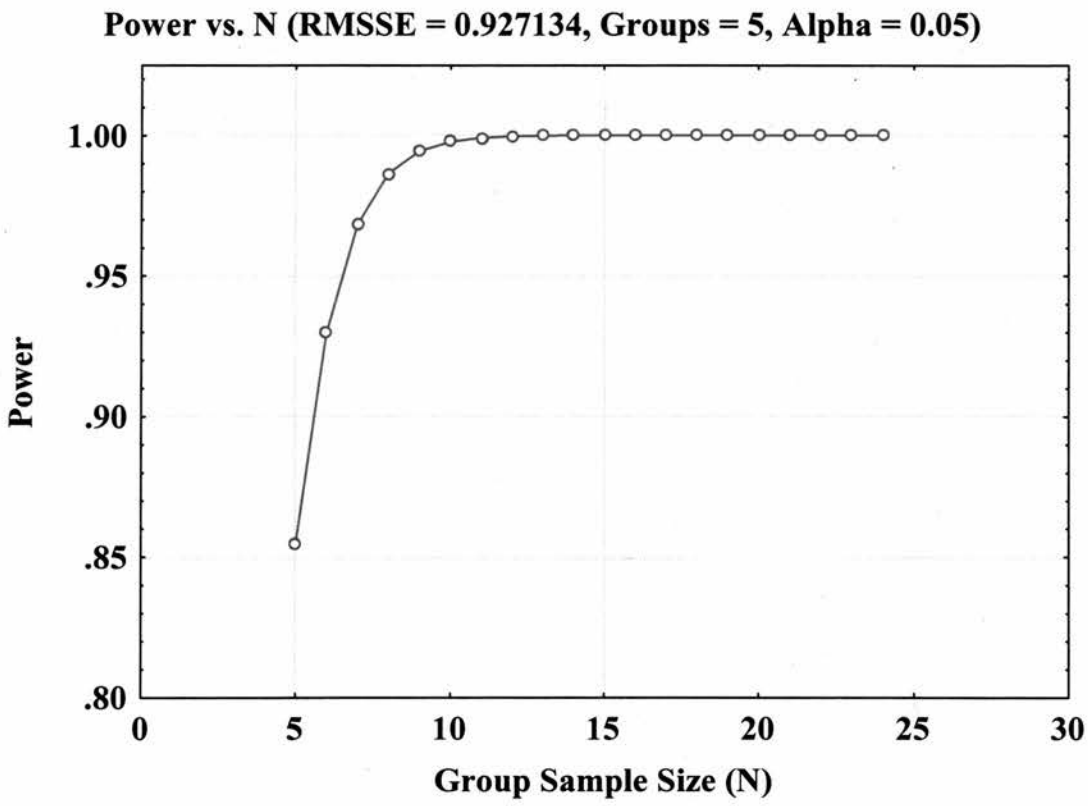


Figure 6

An example of a graph of statistical power against the size of the experimental group. This example was an ANOVA study in which 5 models of repair were compared. Power was computed for $\alpha = 0.05$. In the actual experiments there were 6 animal in each group which gave a power > 0.9.

EXPERIMENTAL APPLICATION OF THE TESTS

The tests described above, variously in combination, were used in the sheep to investigate a number of models of nerve injuries and their surgical repair. These investigations are recorded in Chapters 4 to 8. Taken together, this body of experimental evidence may be used also to evaluate the tests themselves and the animal model. The conclusions drawn from these aspects of the study are recorded in Chapter 9.

CHAPTER 4 — A MULTIMODAL MODEL

THE MEDIAN NERVE IN THE SHEEP

By means of the surgical methods described in Chapter 2 and the tests described in Chapter 3 it was possible to test clinically appropriate surgical models of injury and repair in specific nerves. In human practice, most injured nerves contain both sensory and motor nerve fibres and are described as multimodal. Because of this added complexity in their anatomy it is hypothesized that multimodal nerves would show a poorer outcome after repair than unimodal nerves. This question is considered in Chapter 5. In the present chapter experiments to compare the various models of injury and repair are described. Initially, however a comparison simply of normal and repaired nerves is included.

A GENERAL COMPARISON OF NORMAL & REPAIRED NERVES

In these experiments a population of normal sheep (N = 13) was compared with a population of animals (N= 26) in each of whom the right median nerve had been transected and repaired by means of conventional epineurial suture with 10/0 polyamide (Ethilon, Ethicon U.K.). All of the operations were carried out by a very experienced microsurgeon (M.G.) and it is thus to be supposed that the best possible results might be obtained. The animals were reviewed at 10 months. In each case for greater accuracy in assessing the results 'within-groups variation' was minimized by expressing the measured variables each as a ratio of that obtained in the treated limb divided by that obtained by an identical measurement at the identical site on the opposite limb. This procedure was carried out both in the treated animals and in the

normal controls where, of course, the ratio was expected to be very close to unity. Although every attempt was made to match sheep within groups for size and weight it was inevitable that this was not always possible, especially after the foot-and-mouth epidemic. This strategy of expressing the results in the form of ratios is thus very useful and has the added advantage of allowing multiple findings to be compared on the same set of axes (Figure 1). It is described more fully in Chapter 7 (Fullarton, Glasby, & Lawson 1998; Glasby, Fullarton, & Lawson 1997; Glasby, Fullarton, & Lawson 1998; Lawson & Glasby 1995).

Results

The value of this preliminary study was to determine which variables would be of most potential use in prophesying the outcome of procedures for injury and repair. There were several obvious contenders (Figure 1):

1. CV_{max}
2. Target-muscle mass
3. *TSJ*
4. F_{min}

Of these target-muscle mass is of no clinical use but is helpful in the laboratory. Based upon these findings it may be expected that careful observation of these variables in the models of injury and repair will bear the most fruitful results.

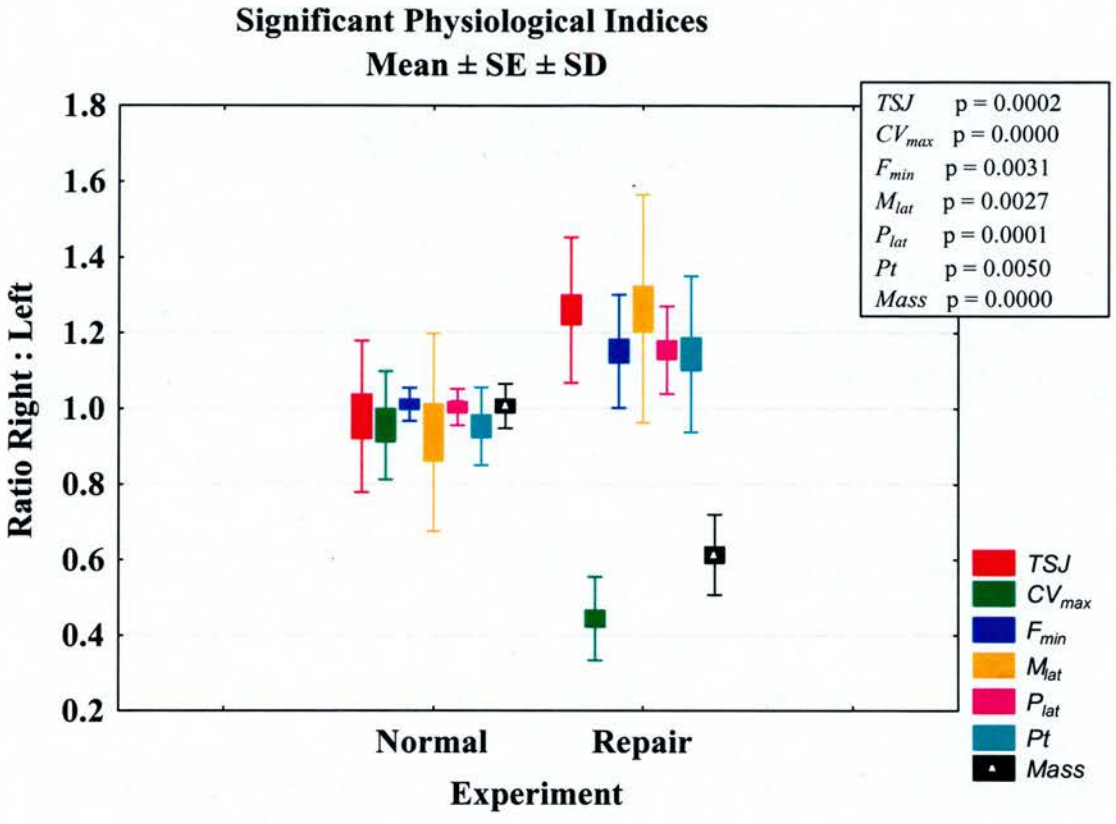


Figure 1

A summary of those physiological indices of recovery which were found to be significant ten months after division and epineurial repair of the median nerve.

Although the above tests were recognized as the likely contenders for forecasting outcome, the entire battery of tests outlined in Chapter 3, including CV_{Dist}, was used in the present study. This was to ensure that when smaller groups were used and when the ratio method was not used, the possible range of tests had been given a fair chance. Although it might be argued that this is a retraction of one's principles, it must be remembered that the majority of work of a similar nature which has been reported in the literature has involved small groups of animals and had relied only on the F test to deal with within-groups variation. It was thus thought appropriate, in the first instance, to perform the evaluative tests as nearly as possible to those already

carried out elsewhere. However in the present study a further evaluation of the tests themselves was inbuilt in the form of power studies.

A COMPARISON OF SPECIFIC SURGICAL MODELS

The following experimental groups were set up as described in Chapter 2 and analysed as described in Chapter 3:

1. Normal controls
2. Neurapraxia
3. Axonotmesis
4. Neurotmesis + repair by epineurial suture
5. Neurotmesis + repair by CRG entubulation
6. Neurotmesis + repair by means of 1 cm reversed full-thickness nerve autograft + epineurial suture.

The animals made a good post-operative recovery and were mobilizing well the day after surgery. There were no incidences of lameness and the animals were able to bear weight on the operated limb without difficulty.

Results

All of the animals were maintained for ten months as described in Chapter 2. They were then re-assessed at terminal experiments conducted as described in Chapters 2 and 3. The mean values for the variables measured in the tests which were carried out are summarized in the following table:

Variable	*Normal*	*Neurapraxia*	*Axonotmesis*	*Neurotmesis + Epineurial Suture*	*Neurotmesis + CRG Entubu-lation*	*Graft*
TSJ (μs)	**8.8**	**10.7**	**10.0**	**10.2**	**9.6**	**10.2**
CV_{max} ($m\ s^{-1}$)	**82.44**	**77.80**	**56.26**	**40.40**	**42.52**	**35.44**
ARP_{min} (ms)	**1.0**	**1.0**	**0.9**	**0.9**	**0.7**	**0.8**
ARP_{max} (ms)	**6.2**	**6.6**	**6.3**	**6.4**	**6.3**	**6.7**
ARP_{range} (ms)	**5.2**	**5.6**	**5.4**	**5.6**	**5.6**	**5.9**
Peak tension (N)	**5.5**	**3.0**	**4.8**	**2.6**	**4.9**	**2.1**
***Time to peak* (ms)**	**48.0**	**43.2**	**39.2**	**46.4**	**41.2**	**37.3**
R½ (ms)	**61.5**	**35.3**	**36.6**	**40.1**	**70.6**	**35.6**
***TT* ∫(mNs)**	**433.0**	**163.7**	**352.4**	**136.6**	**280.5**	**97.6**
***TTI index* (N)**	**3.8**	**2.0**	**3.3**	**2.2**	**3.2**	**1.3**
***Peak tet* (N)**	**22.9**	**22.2**	**22.6**	**17.2**	**22.7**	**17.6**
R½ *tet* (s)	**20.5**	**18.1**	**21.2**	**13.8**	**18.6**	**13.0**
***TT* ∫*tet* (Ns)**	**392.2**	**351.7**	**461.3**	**197.6**	**332.4**	**165.2**
***TTI tet* (N)**	**19.2**	**19.2**	**21.8**	**13.5**	**17.8**	**12.0**
Mass (g)	**9.6**	**9.0**	**7.5**	**5.2**	**5.9**	**5.9**
Axon diameter (μm)	**8.99**	**7.44**	**6.46**	**4.75**	**4.41**	**4.33**
Fibre diameter (μm)	**16.72**	**15.29**	**10.82**	**8.39**	**8.31**	**8.21**
Myelin thickness (μm)	**3.87**	**4.12**	**2.17**	**1.82**	**1.95**	**1.94**
g-ratio	**0.54**	**0.48**	**0.60**	**0.56**	**0.53**	**0.52**

Table 1

The mean values of each of the variables obtained in each of the tests carried out to assess the various models of nerve injury and repair. Values of SD and SEM are omitted for the sake of clarity.

The raw data were 'weeded' as described in Chapter 3 to remove outliers and the residuals calculated for each data column. The residuals were plotted as normal plots and tested for their fit to a normal distribution with the Shapiro–Wilk W test. All of the data were found to be normally distributed and so an F-test was performed using the 'Breakdown & One-way ANOVA' programme in 'Statistica'. The results are shown in Table 2.

	Analysis of Variance (MEDIAN RESULTS weeded) Marked effects are significant at p < .05000							
Variable	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Axon	106.8	5	21.4	14	25	0.58	37.13398	0.000000
Fibre	405.4	5	81.1	36	25	1.45	55.88408	0.000000
Myelin	29.1	5	5.8	4	25	0.14	40.85553	0.000000
G-ratio	0.0	5	0.0	0	25	0.00	9.65258	0.000032
TSJ	19.3	5	3.9	54	31	1.73	2.22227	0.077076
CVmax	14958.8	5	2991.8	4580	30	152.66	19.59702	0.000000
RRP	4.6	5	0.9	33	30	1.10	0.84194	0.530783
ARP	0.4	5	0.1	1	30	0.02	3.41742	0.014610
Pk(ms)	611.0	5	122.2	5836	28	208.43	0.58629	0.710301
Pk(N)	100.5	5	20.1	267	28	9.52	2.11167	0.093550
R/2(ms)	8041.9	5	1608.4	18147	28	648.10	2.48167	0.055624
TT INT	610606.0	5	122121.2	1177408	28	42050.28	2.90417	0.031015
TTIR/2(N)	38.0	5	7.6	109	28	3.89	1.95222	0.117199
Tet(N)	348.9	5	69.8	499	28	17.82	3.91647	0.008111
Tet/2(s)	460.3	5	92.1	1678	27	62.14	1.48147	0.228600
Tet TT INT	413669.8	5	82734.0	639163	27	23672.69	3.49491	0.014521
TeTIR/2(N	474.8	5	95.0	385	27	14.26	6.65982	0.000370
Mass(g)	123.2	5	24.6	94	28	3.35	7.34661	0.000166

Table 2

Computer-generated output for the one-way ANOVA (F test). The values in red are significant at $p<0.05$. The cases in blue, whether significant or not are applicable in the experimental laboratory but not in clinical practice.

The above table shows that only CV_{max} and ARP may be used clinically as general predictors in all cases. This does not rule out CV_{Dist} (see below) as it was not tested here nor the possibility that other tests may be of value where larger differences exist. It would be expected from earlier work that there might not be much difference in outcome between the various groups in which the nerves were transected and between neurapraxia and axonotmesis.

Where the simple comparison of normal and repaired nerves was made (Figure 1) TSJ also showed up significant differences. F_{min}, was not, unfortunately, tested in the present study. The non-significance of TSJ was disappointing as this has consistently been of value in previous studies from our laboratory. It is possible that the group size was too small and indeed the means were all very close and the statistical power was 0.49. In contrast, for CV_{max} the mean velocities showed a progressive decline from normal to the most serious insult of a repair with an autograft and this had a corresponding statistical power of 0.96. Absolute refractory period had a statistical power of 0.69 which, given the test is somewhat more complicated and time-consuming to perform than CV_{max}, would suggest that it is not associated with any advantage.

The tests outlined in blue in Table 2 are, of course of no clinical use. The low p-values for the conventional morphometric tests and for the measurement of muscle mass boosts the already high status of these observations in laboratory studies. The statistical powers for these variables were: fibre diameter 0.99, axon diameter 0.98, and muscle mass 0.86. Myelin sheath diameter and g-ratio are, of course, derived values.

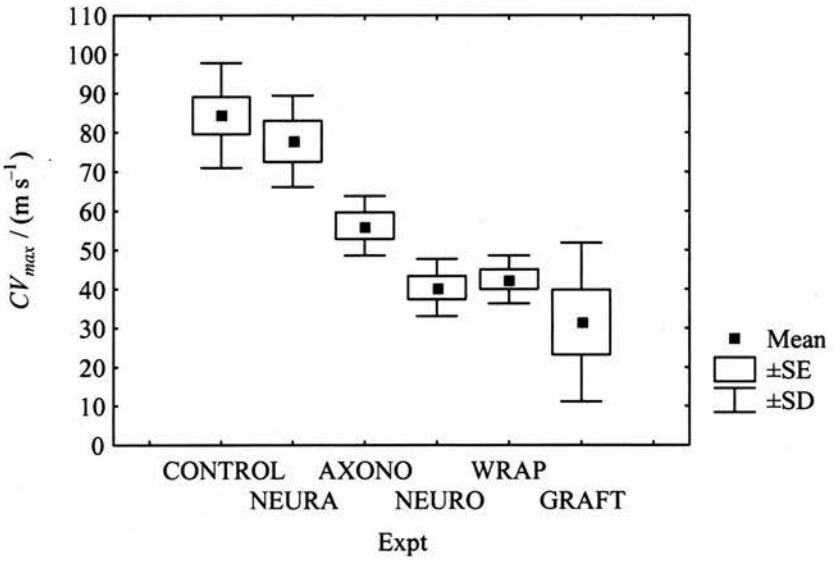
Although some of the variables measured by means of muscle isometric tension studies did have some discriminatory value associated with high statistical power it

seems unlikely, on balance, that much is to be gained from further use of these tests. As they involve a considerable amount of extra time and effort and thereby generate greater costs it is difficult to see what benefit they confer especially as they have no place in clinical practice. As a result of this finding it was decided not to pursue these tests in the assessment of the facial nerve (Chapter 5).

Differences between experimental groups

The remit of the present work was to examine the methods of assessment of nerve injury and repair rather than to comment upon the differences between nerve injuries of varying types and the methods used to correct them. Such is dealt with more fully in a number of publications from EPNRL and from other sources. However the present experiments did permit, albeit in small samples, a statistical comparison of the various injuries and their repair. Given the findings reported above it was only appropriate to progress from the F test to the Scheffé test for the variables CV_{max}, ARP, the morphometric indices and muscle mass. The results of these analyses are summarized in Figures 2, 3 and 4.

Figure 2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90
80
70
60
50
40
30
20
10
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AXONO
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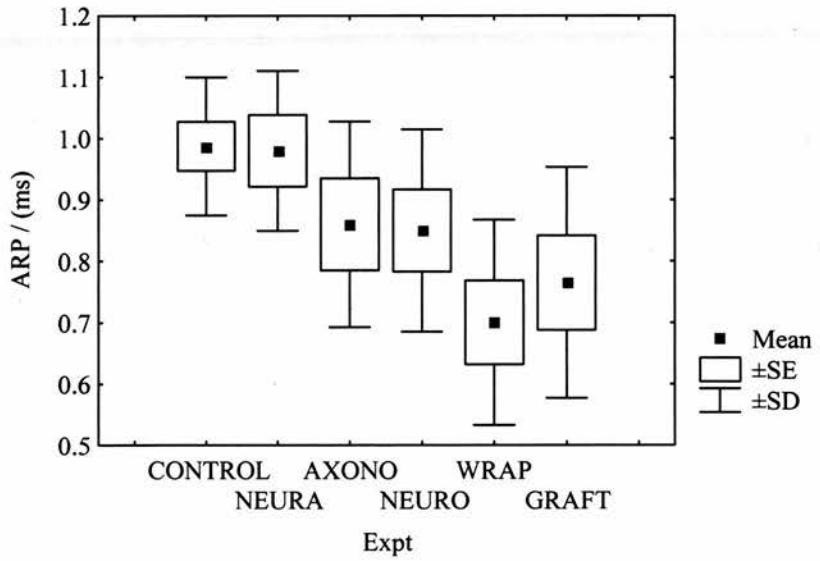
NEURA
NEURO
GRAFT
Expt
Mean
±SE
±SD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1.1
1.0
0.9
0.8
0.7
0.6
0.5
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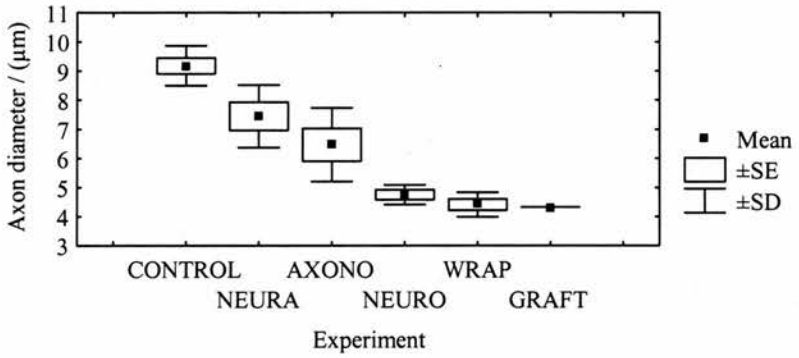
NEURA
NEURO
GRAFT
Expt
Mean
±SE
±SD

Figure 3


Axon diameter / (µm)
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10
9
8
7
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5
4
3
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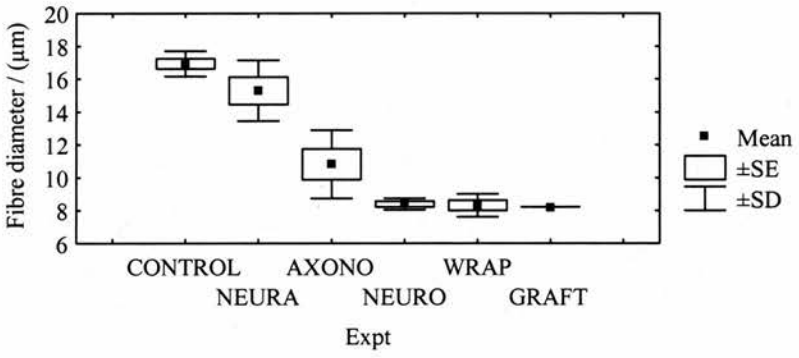
Experiment
Mean
±SE
±SD


Fibre diameter / (µm)
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18
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14
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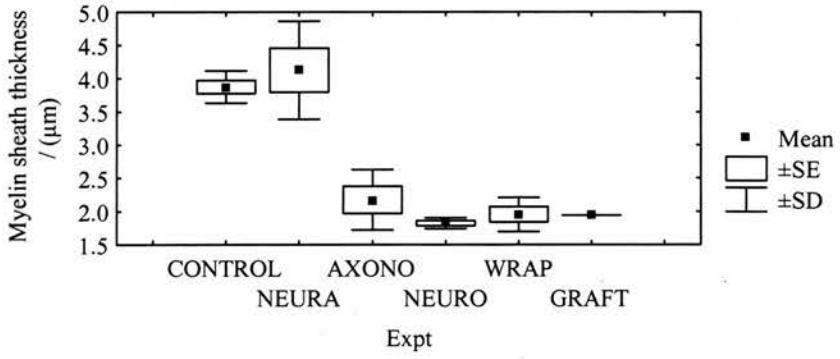
6
CONTROL
AXONO
WRAP
NEURA
NEURO
GRAFT
Expt
Mean
±SE
±SD


Myelin sheath thickness / (µm)
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4.0
3.5
3.0
2.5
2.0
1.5
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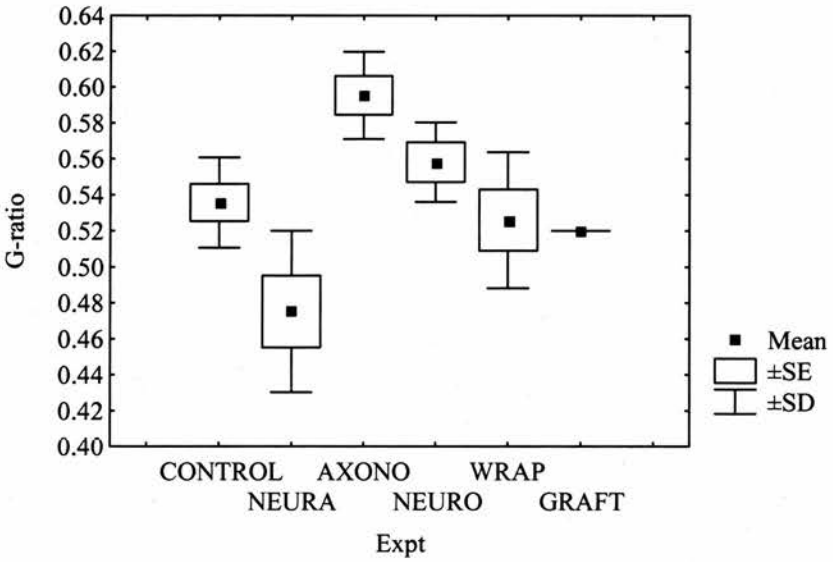
WRAP
NEURA
NEURO
GRAFT
Expt
Mean
±SE
±SD

Figure 4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0.60
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0.56
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0.52
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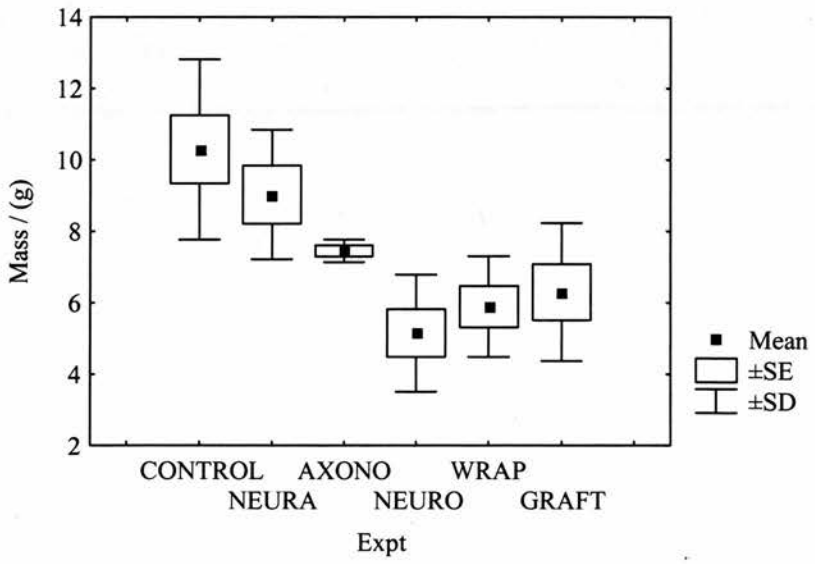
0.48
0.46
0.44
0.42
0.40
CONTROL
NEURA
AXONO
NEURO
WRAP
GRAFT
Expt
Mean
±SE
±SD


Mass / (g)
14
12
10
8
6
4
2
CONTROL
NEURA
AXONO
NEURO
WRAP
GRAFT
Expt
Mean
±SE
±SD

Once more the value of CV_{max} as a discriminator was obvious though it was not effective in distinguishing between the various models which involved transecting injuries. What was important here is that it did distinguish between neurapraxia and axonotmesis and it did correlate with the results of morphometric analysis. This is encouraging and extremely convenient as CV_{max} is the most readily available and widely understood neurophysiological test available. On the basis of the results obtained in this thesis it is doubtful whether there is anything to be gained in the follow-up of nerve injuries by attempting to use any other supposedly objective test. Of course, the problem is that CV_{max} is not used widely enough in clinical follow-up especially in the United Kingdom and it is only very rarely used sequentially — where it is most useful — to track recovery.

CV_{Dist}

The importance of CV_{max} in assessing nerve injuries raises the question of whether the measurement of CV_{Dist} can offer further useful information. In theory it should as CV_{Dist} makes a quantitative statement about the size of the conducting fibres, in particular those which are not contributing to CV_{max}.

Each experimental group contained six animals. There is no means of performing statistical tests on what is essentially a distribution graph. Thus the individual CV_{Dist} graphs for each group are displayed together on one page. The percentage of fibres conducting impulses was the ordinate and the range of conduction velocities across which the nerve was conducting was abscissa. As the group with nerve autografts was part of another study, it was impossible, for technical reasons, to use these animals in order to measure CV_{Dist}.

Figure 5 shows the six CV_{Dist} graphs for the normal control group: they all had a bimodal distribution.

Figure 6 shows five CV_{Dist} graphs for the neurapraxia model for the median nerve. Graph four is missing as this animal died of an unrelated cause. All five graphs had a bimodal distribution. As far as it was possible to determine, there was no marked difference between the neurapraxia group and the normal group.

Figure 7 shows five CV_{Dist} graphs for the axonotmesis model for the median nerve. Graph six is missing as this animal also died. All the graphs had a bimodal distribution with a general shift to the left when compared with normal controls and with the neurapraxia model.

Figure 8 shows the six CV_{Dist} graphs for neurotmesis and repair by epineurial interrupted suture. Graphs one to four and graph six had a bimodal distribution but in graph five there was only one peak. The graphs were shifted **further** to the left when compared with the normal control, neurapraxia and axonotmesis models.

Figure 9 shows the six CV_{Dist} graphs for the neurotmesis and repair by CRG entubulation. The bimodal distribution was preserved in all six graphs which were shifted to the left as in Figure 8. There was no obvious difference between the neurotmesis and suture repair and the neurotmesis and wrap repair models.

Median Nerve
Normal Control

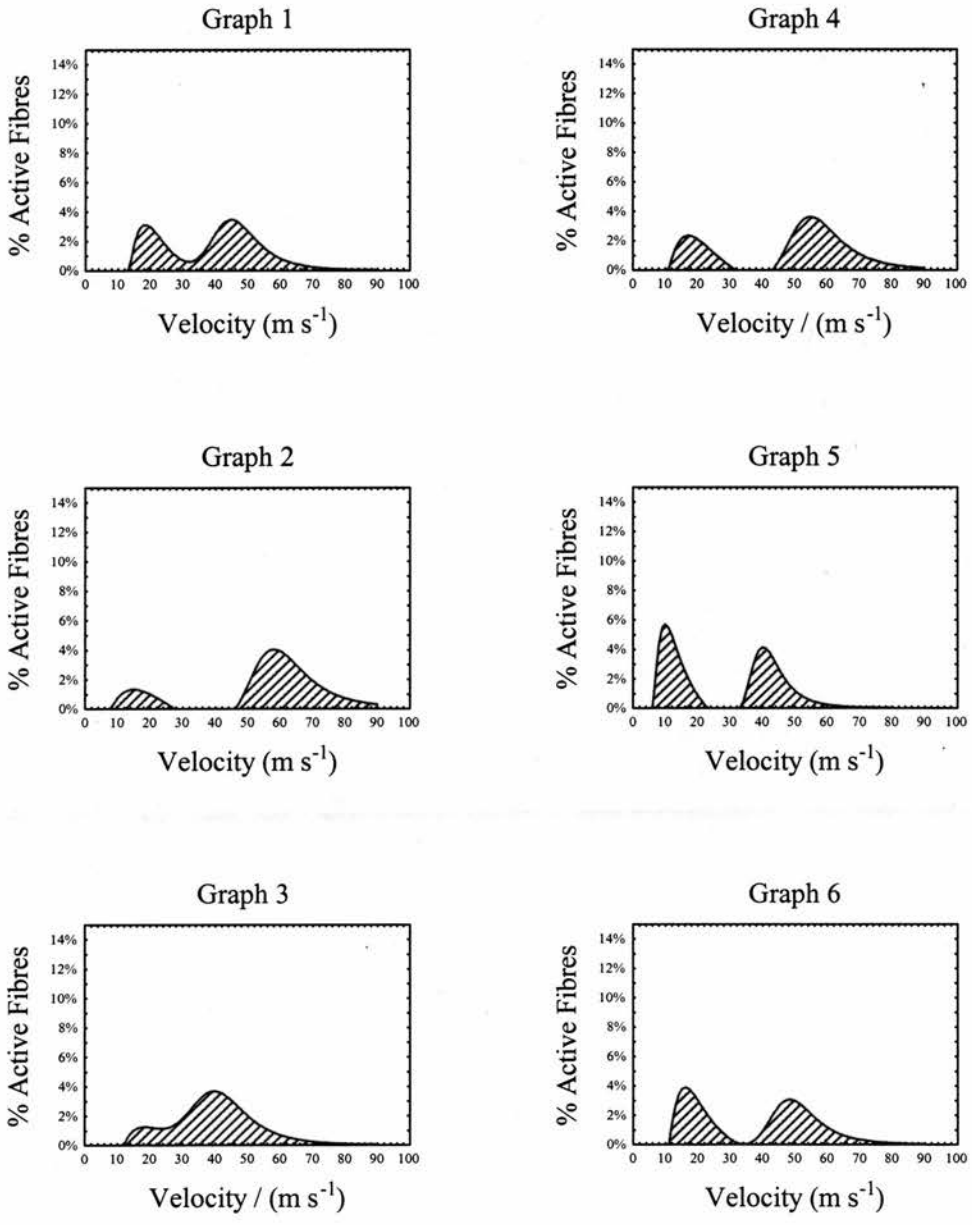


Figure 5

Median Nerve
Neurapraxia

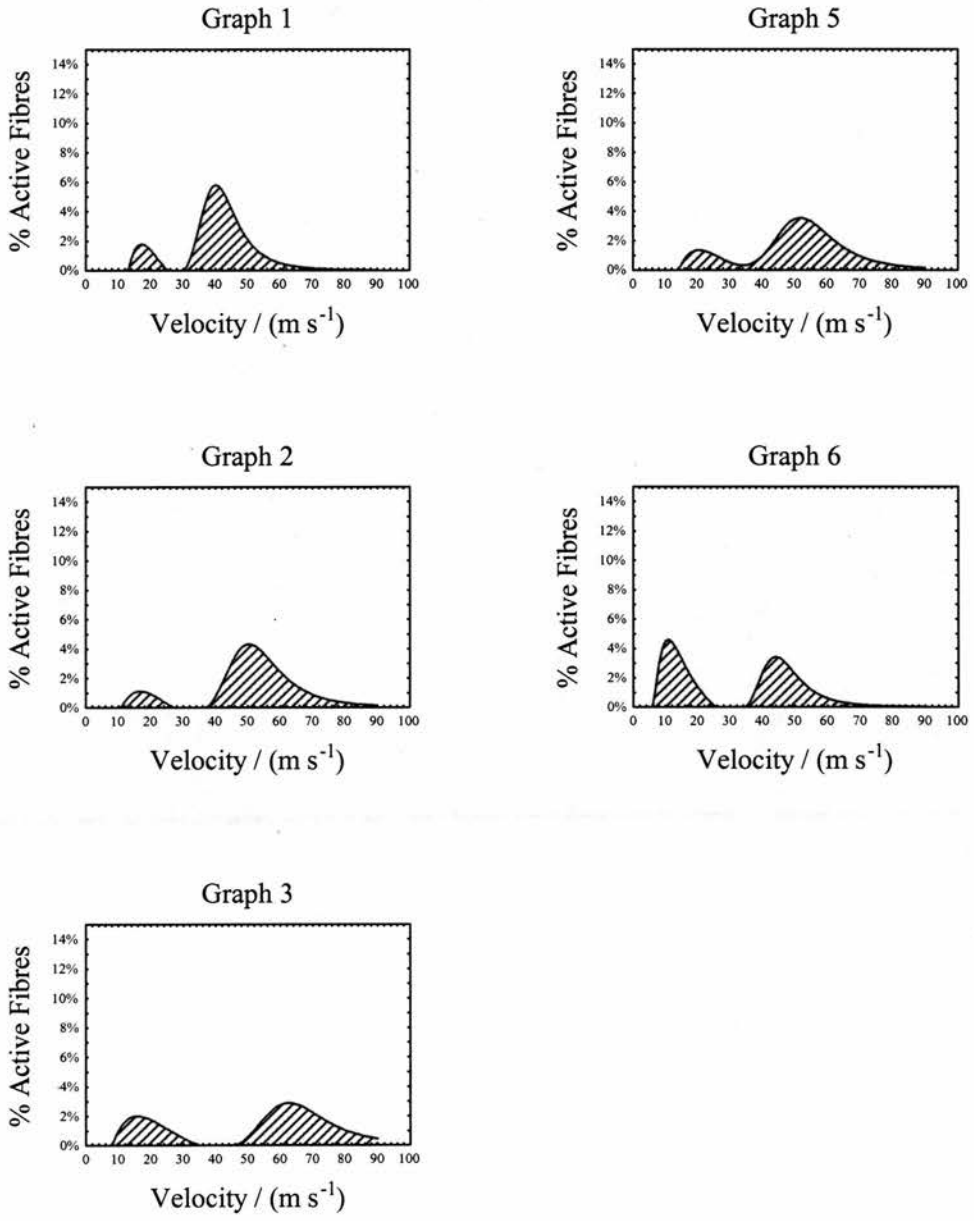


Figure 6

Median Nerve
Axonotmesis

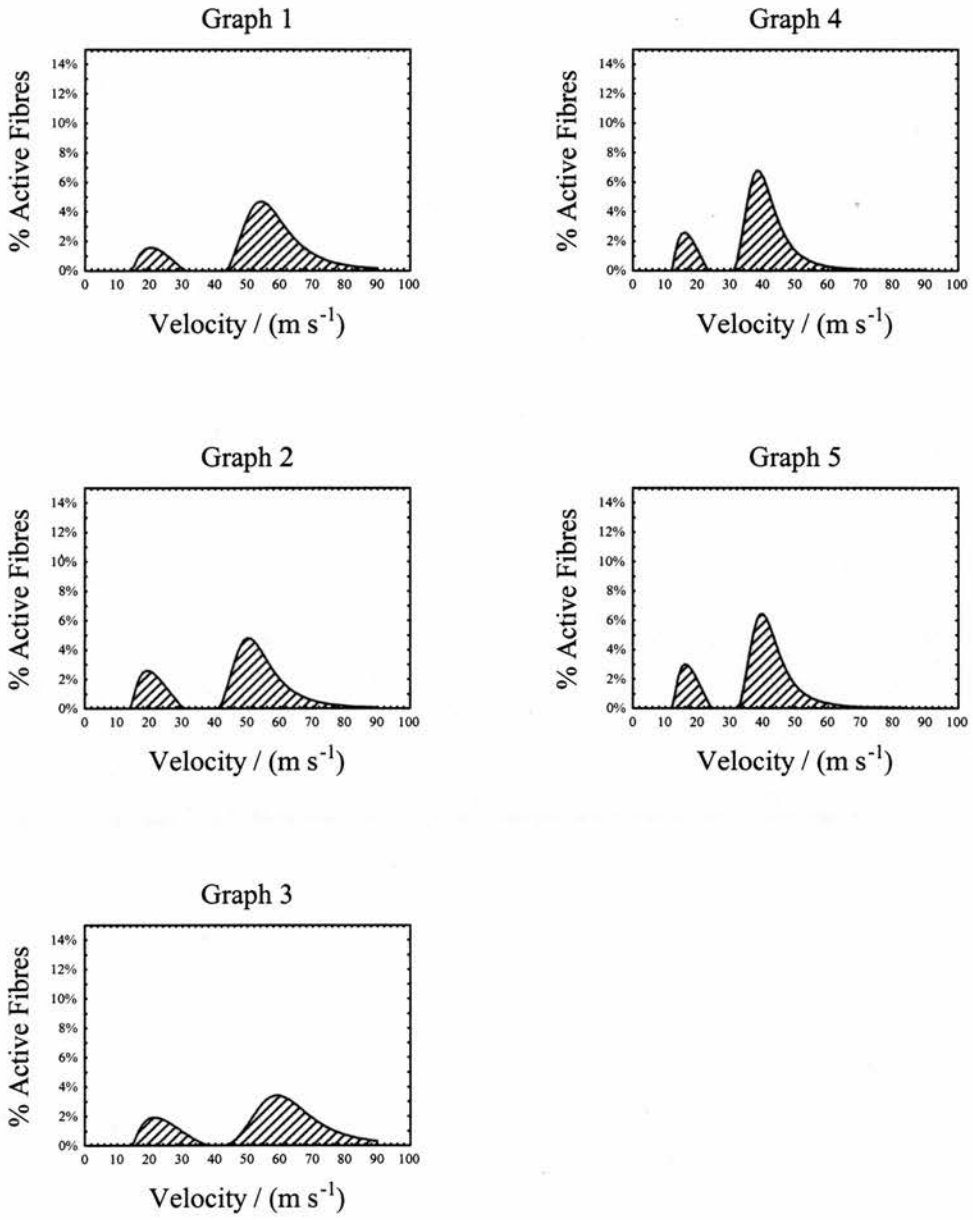


Figure 7

Median Nerve
Neurotmesis + Epineurial Suture

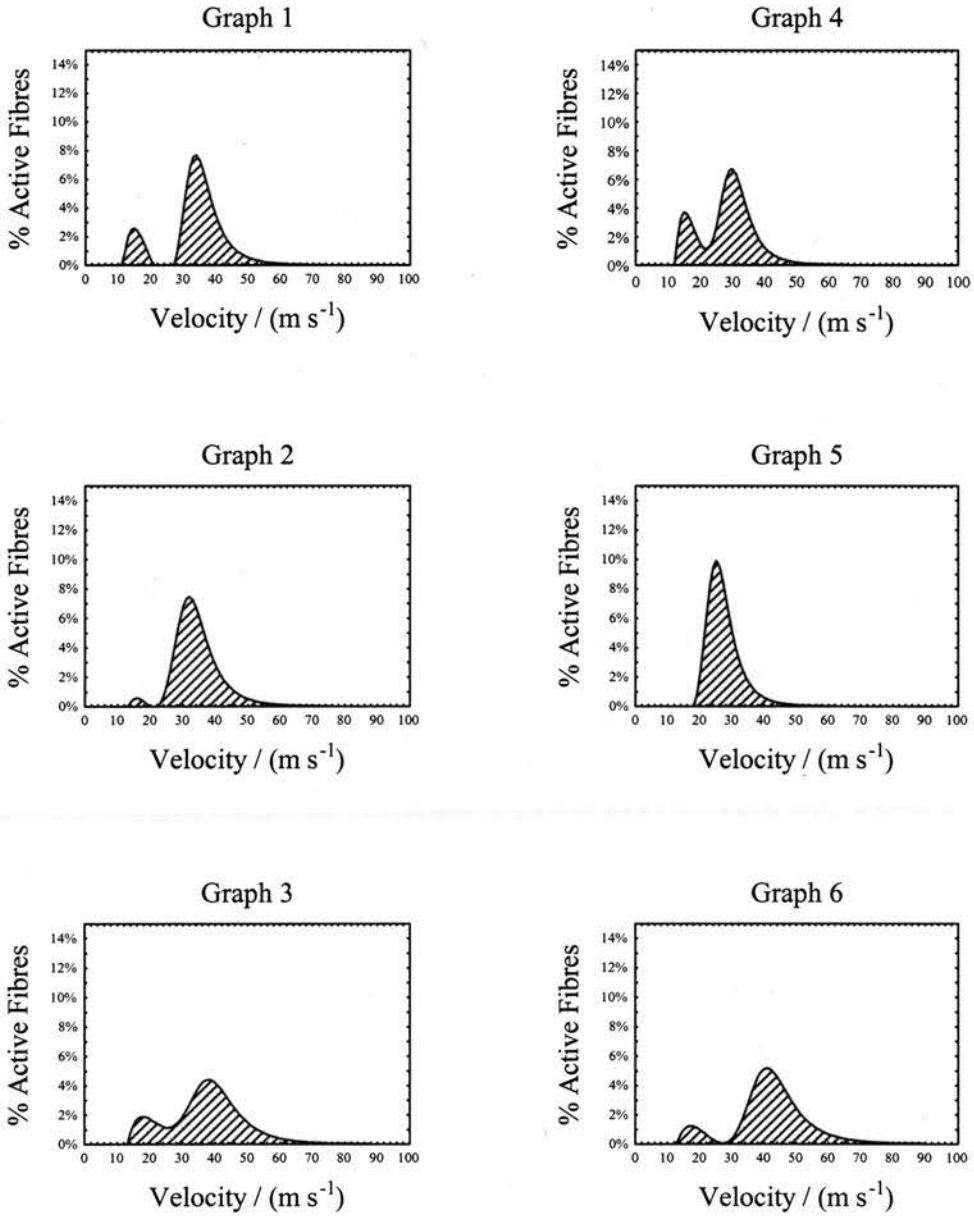


Figure 8

Median Nerve
Neurotmesis + CRG Entubulation

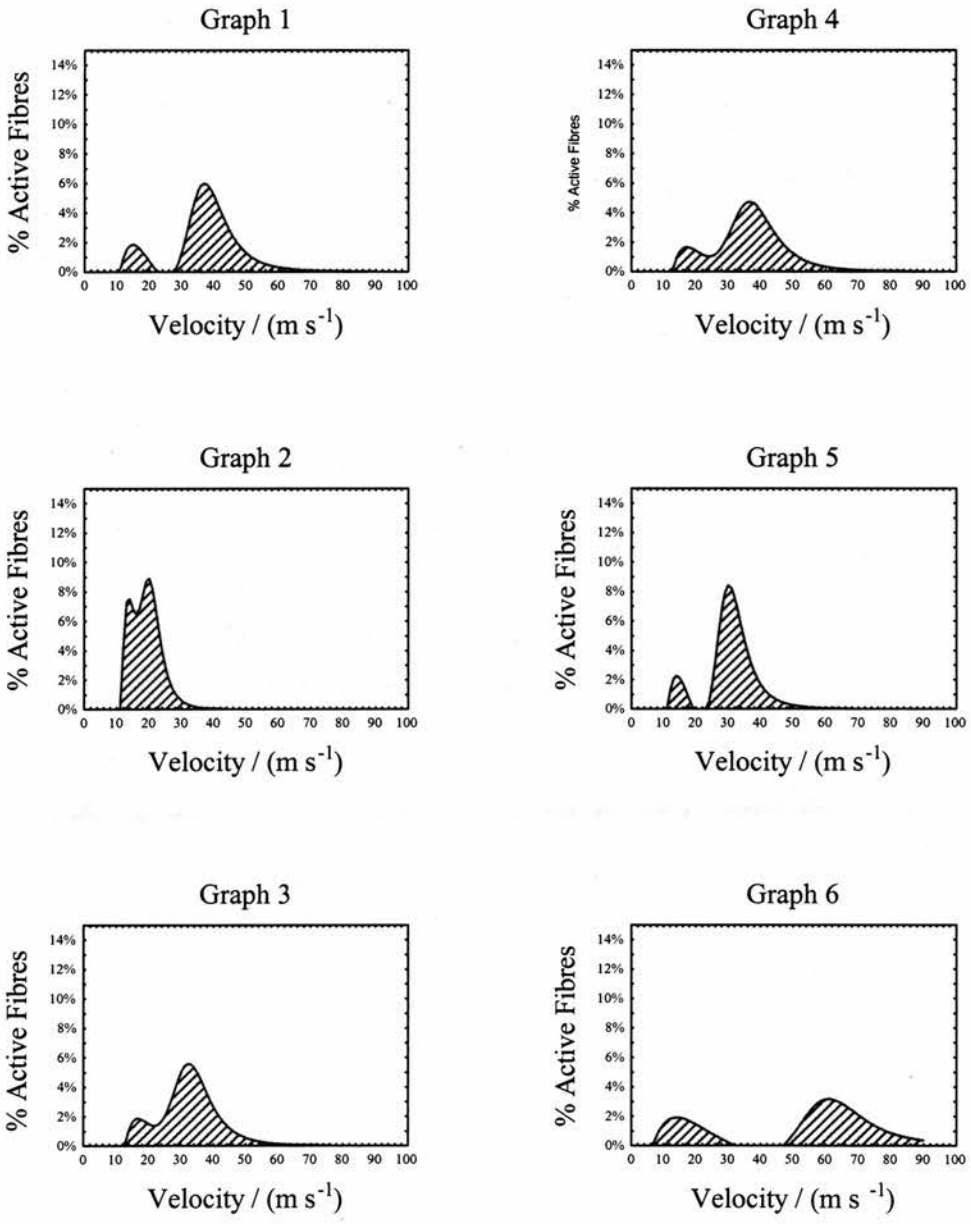


Figure 9

Conclusions

The findings reported in this chapter strongly support the use of CV_{max} as a routine index of the events taking place in nerve injury and after its repair. This measurement correlates well with those findings from invasive tests which themselves are good markers of progress and which can disclose differences in outcome. The two additional clinically available tests which may be useful adjuncts to CV_{max} are ARP and CV_{Dist}. The former, though it has appropriate discriminatory power, does not add sufficiently to the armamentarium to be worth the additional effort, time and cost incurred by its use.

CV_{Dist} is more equivocal. Its drawback is that is it does not provide a simple quantity which may be compared among individuals and at different times. It does, however, tell us a lot about what is taking place in the recovering nerve. As mentioned in Chapter 6 its real value may be in studying neuropathies neurotoxicity and perhaps it may be of help in differentiating cases of neurapraxia and axonotmesis when there is a real need to know precisely which is present. For transecting injuries it is too non-specific but may, if used over time, be helpful in following-up regeneration. Its value as a clinical test is further discussed in Chapter 6.

Although small groups have been used, the results for each of the models considered here have precisely mirrored what has been found in other studies and this lends weight to what we know about nerve regeneration after repair

Finally, as a result of these studies, the sheep emerges as a good model for nerve injury and repair and a good medium in which to apply the various tests which were tried out.

CHAPTER 5 — A UNIMODAL MODEL

THE FACIAL NERVE IN THE SHEEP

THE facial nerve has been the subject of extensive work at EPNRL over a number of years (Drew et al. 1995; Glasby et al. 1995; Glasby, Mountain, & Murray 1993; Glasby & Sharp 1992; Kelly 2001; Mountain et al. 1993). From a purely theoretical point of view a unimodal nerve should show a better outcome after transecting injury and repair though not after injury which maintains continuity. This is because the regeneration by pioneering axons down Schwann cell basement membrane tubes is a random process. If there are fewer modalities to enlist in the turmoil, then there should be fewer re-wiring 'mistakes' and fewer pioneering axons will either make inappropriate connections or fail to make connections and die-back.

The present experiments were part of a series to assess the outcome of injury and repair of the facial nerve *per se*, using the same models as in the case of the median nerve but clearly, given that the experimental protocol was identical, it was possible as a by-product to consider the differences between a typical unimodal nerve and a typical multimodal nerve.

EXPERIMENTAL PROTOCOL

The experimental groups were the same as for the experiments described in Chapter 4 except that the model nerve was the buccal branch of the facial nerve. The groups were thus:

7. Normal controls
8. Neurapraxia
9. Axonotmesis

10. Neurotmesis + repair by epineurial suture
11. Neurotmesis + repair by CRG entubulation
12. Neurotmesis + repair by means of 1 cm reversed full-thickness nerve autograft + epineurial suture.

In view of the findings in Chapter 4 The variables which were tested were:

1. CV_{max}
2. CV_{Dist}
3. TSJ
4. ARP
5. Muscle mass
6. Axon diameter
7. Fibre diameter.

Myelin sheath thickness and g-ratio were calculated. Although TSJ was not found to be adequately discriminatory in the median nerve its p-value was close to $p = 0.05$ and it was possible that the small group size had had an effect. For this reason it was decided to assess TSJ again.

Surgical approach to buccal branch of the the facial nerve

The animal was placed in the right lateral position and the left side of its face was shaved using electric clippers. The facial nerve and vein could be palpated through the skin and their course was marked using indelible ink. Some of the animals with large curly horns required removal of the tip of the left horn to allow correct positioning of the microscope vertically above the site of nerve.

The operation site was prepared with povidone-iodine solution (Betadine, Seton Healthcare, England) and covered with sterile surgical drapes. The skin was incised along the previously marked course of the nerve. Bleeding points were coagulated

using bipolar diathermy and small skin flaps were raised. These were reflected and secured in position with stay-sutures. Connective tissue was divided using sharp dissection to expose the underlying facial nerve between its emergence from the parotid gland and its passing deep to the masseter muscle. The nerve was carefully dissected from the adjacent facial vein. A nerve stimulator was used in some cases to aid location of the nerve.

After the appropriate injury and repair procedure (see Chapter 2) had been carried out and after ensuring haemostasis, the incision was closed. Interrupted sutures of 4/0 polyglactin (Vicryl, Ethicon, UK) were placed in the subcutaneous tissue. The skin was closed with a continuous subcuticular suture also of 4/0 polyglactin. An antiseptic dressing spray was applied to the wound (Opsite, Smith and Nephew, England).

As a result of this procedure the animals had an obvious left-sided facial weakness in the immediate post-operative period. However, in all cases, this had resolved by the time of the second procedure. The animals were observed to be eating normally the day after surgery. One of the sheep developed a small wound effusion, however, this did not become infected and resolved spontaneously.

Experimental Assessment

In most respects surgery and diagnostic the tests were carried out exactly as described in Chapters 2 and 3.

Exposure and placement of electrodes

The left side of the sheep's face and its nasal bridge were shaved using electric clippers. The skin was cleaned with alcohol to remove lanolin present on the skin as

this can hinder the attachment of surface recording electrodes and is an effective dielectric.

The ground electrode was positioned on the skin over the bony nasal bridge. Efficacy of conduction was improved with a small amount of electrode paste (Ten 20, Weaver & Co., Aurora, USA). Surface recording electrodes were similarly attached over the zygomaticus muscle which was located by palpation near the snout of the animal. This muscle was chosen for the assessment as it is uniquely supplied by the facial nerve. The cathode was positioned over the motor point (see Chapter 2) of the muscle and the reference electrode 1cm distally.

The facial neurovascular bundle was more difficult to palpate at the second procedure owing to the presence of to scar tissue from the first operation. A skin incision was made parallel and slightly rostral to the pre-existing scar. This approach was found to avoid the worst of the scar tissue whilst still being close to the previously located facial nerve. No attempt was made to dissect around the area of the nerve where the previous surgery had been performed as the nerve was surrounded by a dense mass of scar tissue.

The nerve was dissected proximal and distal to the site of injury. A nerve stimulator was used in those cases where location of the nerve proved difficult. The least amount of dissection was performed as was required to allow placement of the electrodes. This was to minimize disruption of the blood supply, avoid the nerve's drying out and to keep it warm. The maximum conduction velocity of a nerve been is known to decrease with decreasing temperature (de Jesus, Hausmanowa-Petrusewicz, & Barchi 1973; Johnson & Olsen 1960). The areas of exposed nerve were kept moist with saline-soaked gauze swabs.

The facial nerve was dissected proximally to 1cm from its exit from the parotid gland. Previous experiments had shown that it was critical at this time not to broach the parotid sheath nor injure the gland in any way lest leakage of parotid secretion occur. The presence of even the smallest amount of this secretion in the vicinity of the nerve had the effect of making the nerve inexcitable. This effect was presumably due to the high $[K^+]$ of parotid secretion which caused hyperpolarization of the axonal membrane. A bipolar (proximal) stimulating electrode (proximal anode and distal cathode), was placed under the nerve at this site.

Distally, the buccal nerve was located by dividing the masseter muscle. It was dissected free from surrounding structures and a (distal) bipolar stimulating electrode was applied. Where possible the maximum distance between the stimulating electrodes was achieved as nerve conduction studies are more accurate over longer distances (Kimura 2001).

TSJ, CV_{max}, CV_{Dist}, ARP, muscle mass, muscle morphometry

Assessment of these variables was carried out as described in Chapter 3 for the median nerve but using the facial nerve set-up described above.

RESULTS FOR MODELS OF INJURY AND REPAIR IN THE FACIAL NERVE

All of the animals were maintained for ten months after the first operative procedure and then re-assessed in terminal experiments. Figure 1 is a summary of the mean values for the all variables which were measured except for CV_{Dist} :

Variable	*Normal*	*Neurapraxia*	*Axonotmesis*	*Neurotmesis + Epineurial Suture*	*Neurotmesis + CRG Entubu-lation*	*Graft*
TSJ (μs)	**8.4**	**9.7**	**8.5**	**8.8**	**8.7**	**8.8**
CV_{max} ($m\ s^{-1}$)	**71.3**	**51.5**	**38.2**	**35.9**	**38.0**	**36.5**
ARP_{min} (ms)	**0.9**	**1.0**	**1.2**	**1.2**	**1.1**	**0.9**
ARP_{max} (ms)	**5.7**	**6.4**	**6.4**	**5.8**	**6.0**	**5.1**
ARP_{range} (ms)	**4.7**	**5.4**	**5.2**	**4.6**	**4.9**	**4.2**
Mass (g)	**2.1**	**2.8**	**2.5**	**2.3**	**2.2**	**2.0**
Axon diameter (μm)	**7.2**	**6.3**	**6.3**	**5.8**	**6.1**	**5.6**
Fibre diameter (μm)	**12.6**	**11.1**	**10.3**	**9.5**	**10.2**	**8.3**
Myelin thickness (μm)	**2.7**	**2.4**	**2.0**	**1.9**	**2.0**	**1.6**
g-ratio	**0.6**	**0.6**	**0.6**	**0.6**	**0.6**	**0.6**

Table 1

The mean values of each of the variables obtained in each of the tests carried out to assess the various models of nerve injury and repair in the facial nerve. Values of SD and SEM are omitted for clarity.

The raw data were 'weeded' as described in Chapter 3 to remove outliers and the residuals calculated for each data column. The residuals were plotted as normal plots and tested for their fit to a normal distribution with the Shapiro–Wilk W test. All of the data were found to be normally distributed and so an F-test was performed using the 'Breakdown & One-way ANOVA' programme in 'Statistica'. The results are shown in Table 2.

Variable	Analysis of Variance (FACIAL RESULTSweeded) Marked effects are significant at p < .05000							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
TSJ	7.097	5	1.419	50.249	34	1.47791	0.96041	0.455577
CVmax	8804.335	5	1760.867	3228.396	34	94.95283	18.54465	0.000000
ARP	0.421	5	0.084	1.703	35	0.04866	1.73208	0.153041
Mass	2.887	5	0.577	7.977	35	0.22792	2.53332	0.046554
Axon	19.913	5	3.983	16.672	36	0.46311	8.59965	0.000020
Fibre	87.472	5	17.494	40.279	36	1.11886	15.63596	0.000000
Myelin	6.264	5	1.253	3.591	36	0.09975	12.56001	0.000000
g-ratio	0.015	5	0.003	0.052	36	0.00144	2.08342	0.090150

Table 2

Computer-generated output for the one-way ANOVA (F test).

The values in red are significant at $p<0.05$.

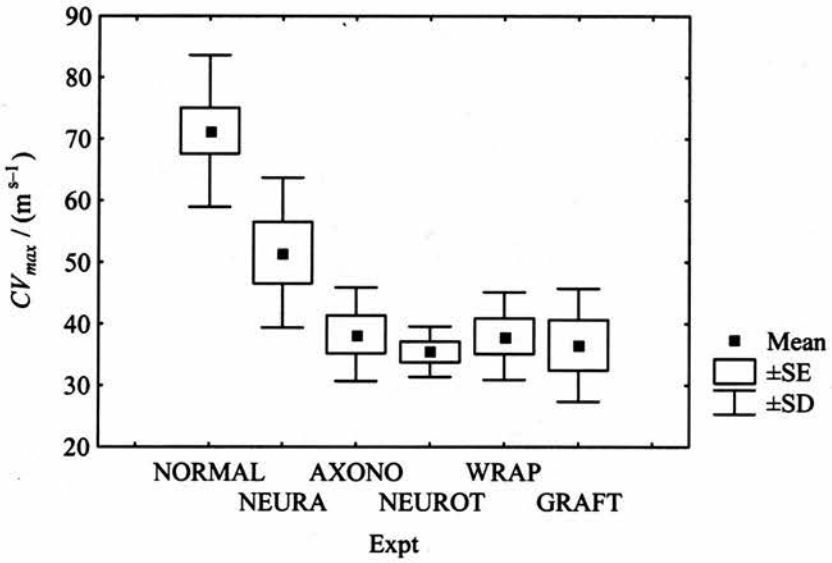
The only test which emerges as clinically useful is CV_{max} though CV_{Dist} was not considered here. Again the result for TSJ was disappointing as was that for ARP. However the finding that ARP was not significant here supports the caveats made for its use in Chapter 4.

The statistical powers obtained here were as follows: CV_{max} = 0.89, Axon diameter = 0.83, Fibre diameter = 0.90, again these are good indications of the discriminative properties of these variables.

Differences between experimental groups

As in Chapter 4 these may be summarized graphically as follows:

Figure 1


90
80
70
60
50
40
30
20
CV_{max} / (m s^{-1})
NORMAL
AXONO
WRAP
NEURA
NEUROT
GRAFT
Expt
Mean
±SE
±SD

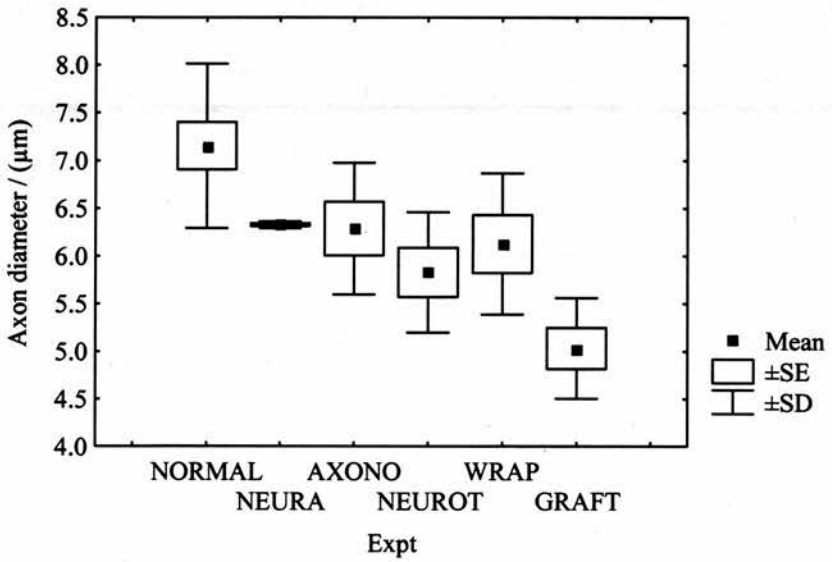
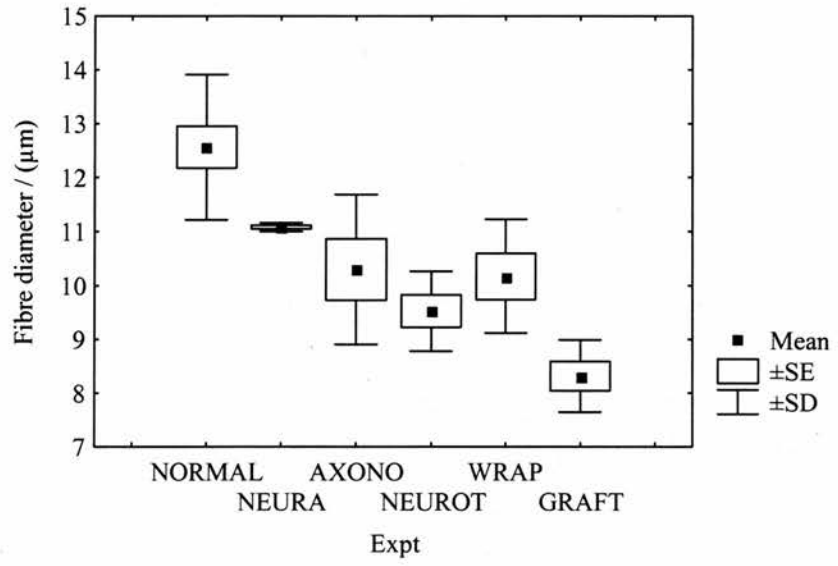
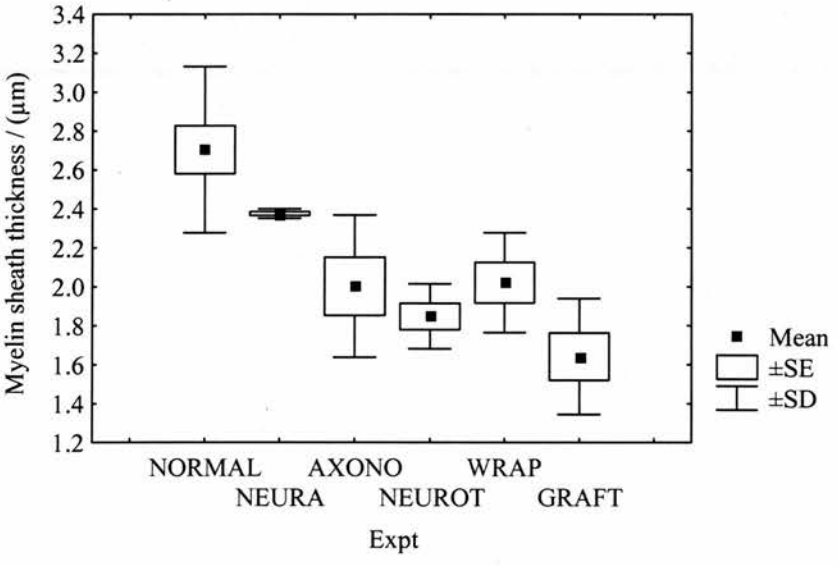

8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
Axon diameter / (µm)
NORMAL
AXONO
WRAP
NEURA
NEUROT
GRAFT
Expt
Mean
±SE
±SD

Figure 2





CV_{Dist}

Each experimental group contained six animals, the corresponding six CV_{Dist} graphs are displayed on one page. The percentage of fibres conducting impulses was

represented on the ordinate whilst the range of active conduction velocities was represented on the abscissa.

Figure 3 shows the six CV_{Dist} graphs for the normal control group for the facial nerve. All six graphs had a bimodal distribution.

Figure 4 shows the six CV_{Dist} graphs for the neurapraxia model for the facial nerve. Again all six graphs had a bimodal distribution. The graphs were shifted to the left when compared with normal controls.

Figure 5 shows the six CV_{Dist} graphs for axonotmesis. Again all six graphs had a bimodal distribution and were shifted to the left compared with the normal controls. Graphs 2, 3 and 5 were shifted to the left compared with the neurapraxia model.

Figure 6 shows the six CV_{Dist} graphs for the neurotmesis and epineurial suture model for the facial nerve. Graphs 1 to 5 had a bimodal distribution but in graph six there was only a suggestion of a second peak. The graphs were shifted to the left compared with the normal control model, neurapraxia model and axonotmesis model.

Figure 7 shows the six CV_{Dist} graphs for neurotmesis + CRG entubulation. The bimodal distribution was preserved in all six graphs. The graphs were shifted to the left when compared with the normal control model, the neurapraxia model and the axonotmesis model. There was no obvious difference between the neurotmesis + epineurial suture model and the neurotmesis + entubulation model.

Figure 8 shows five CV_{Dist} graphs for the neurotmesis + nerve autograft model for the facial nerve. Graph two is missing as this animal died. All five graphs had a bimodal distribution. The graphs were found to be shifted **further** to the left when compared with all the other models of nerve injury.

Facial Nerve
Normal Control

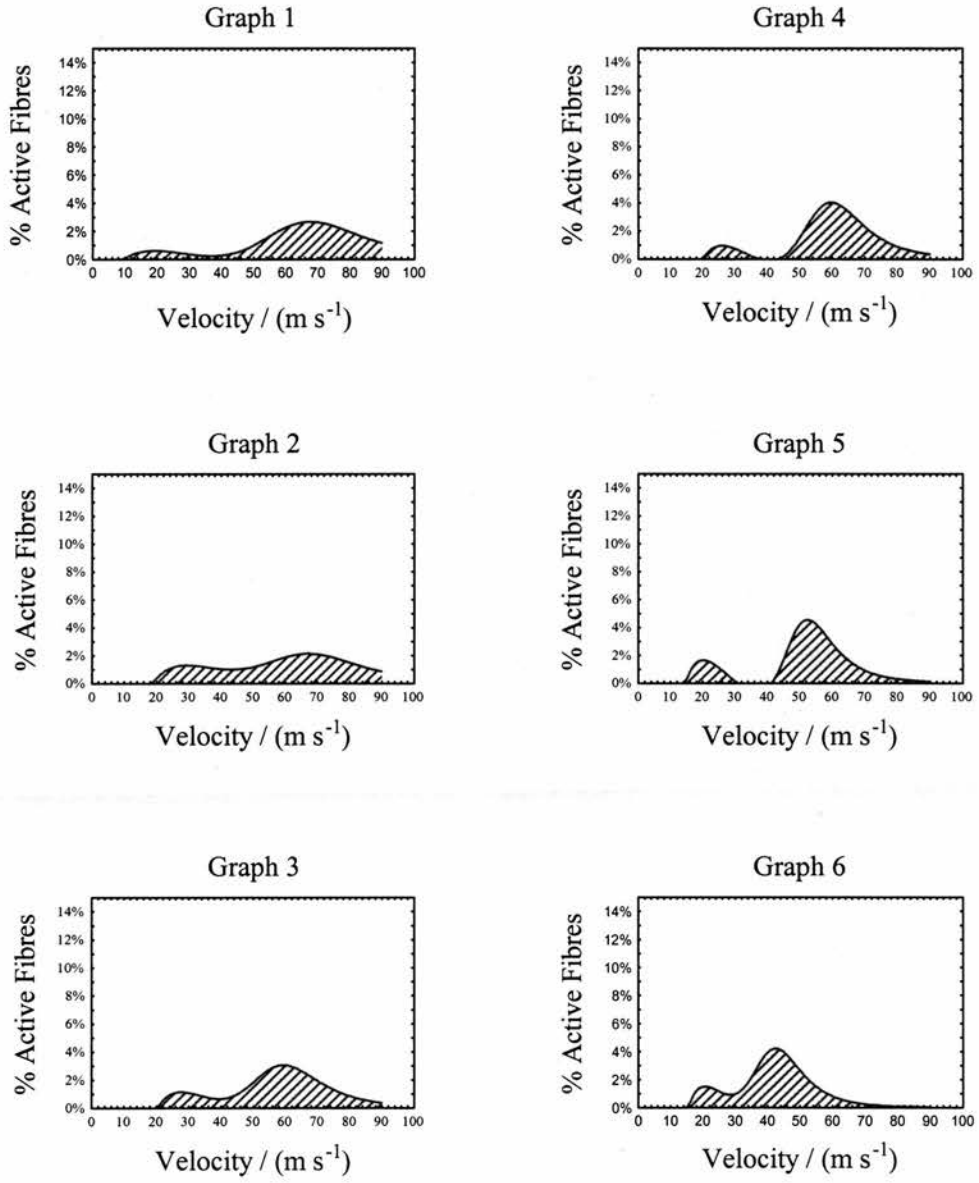


Figure 3

Facial Nerve
Neurapraxia

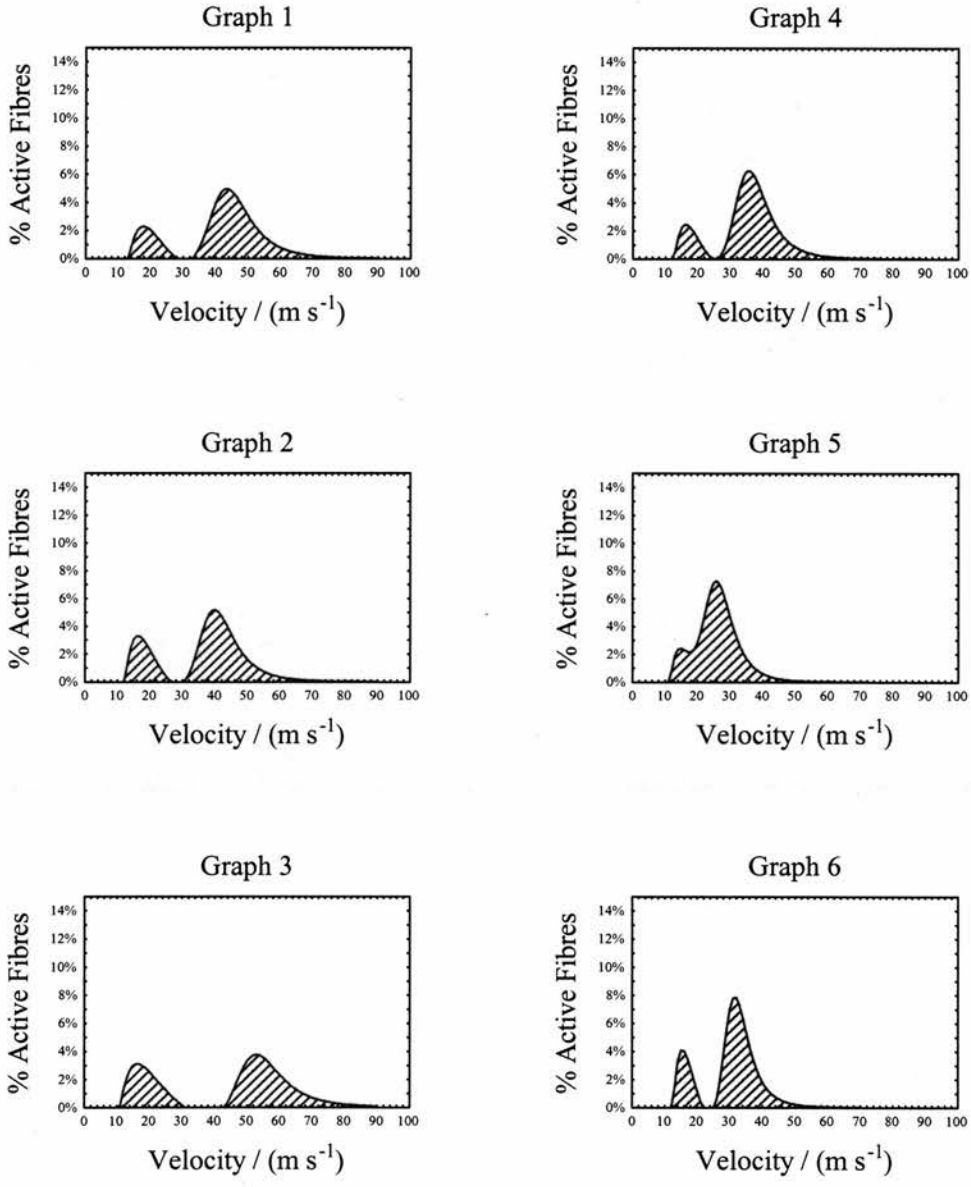


Figure 4

Facial Nerve
Axonotmesis

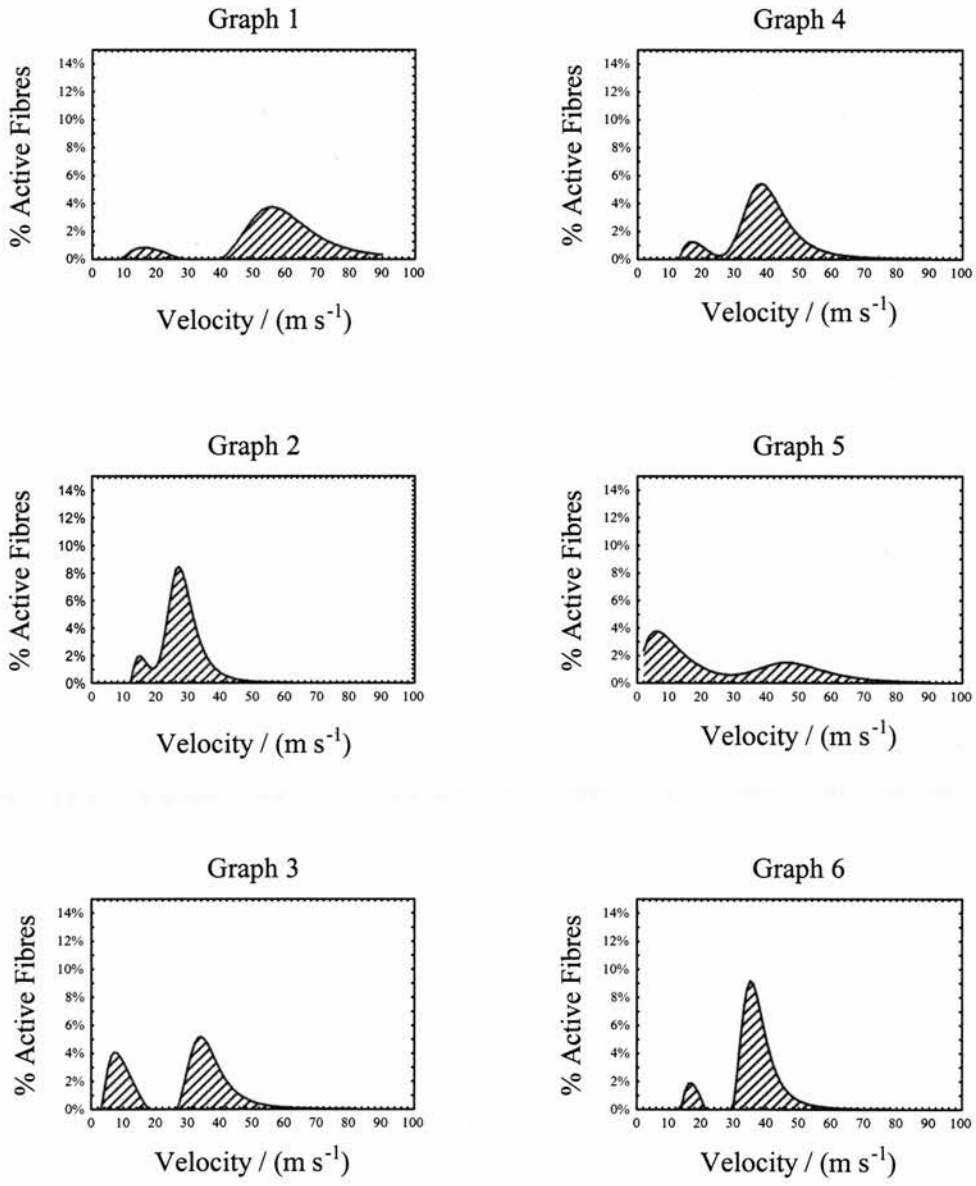


Figure 5

Facial Nerve
Neurotmesis + Epineurial Suture

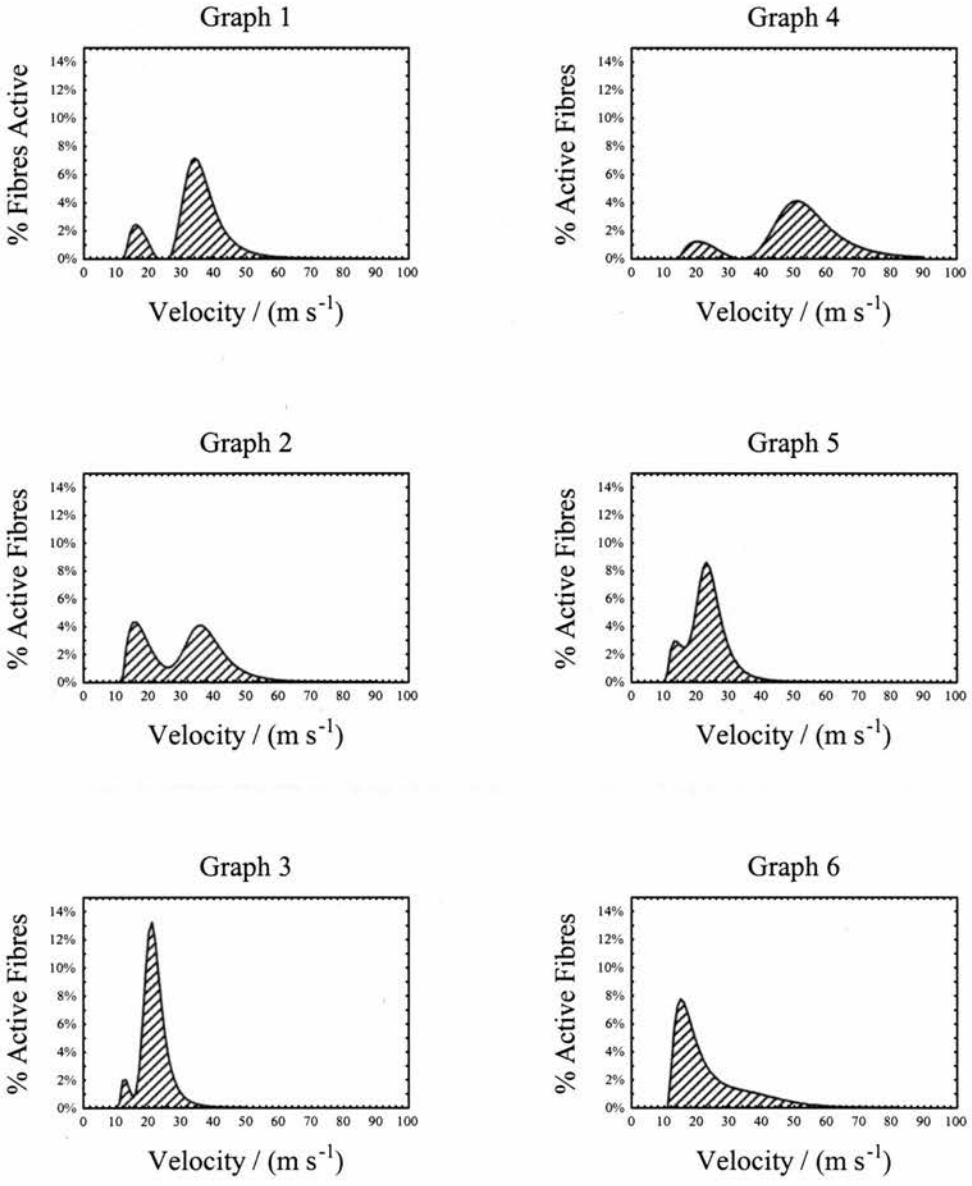


Figure 6

Facial Nerve
Neurotmesis + CRG Entubulation

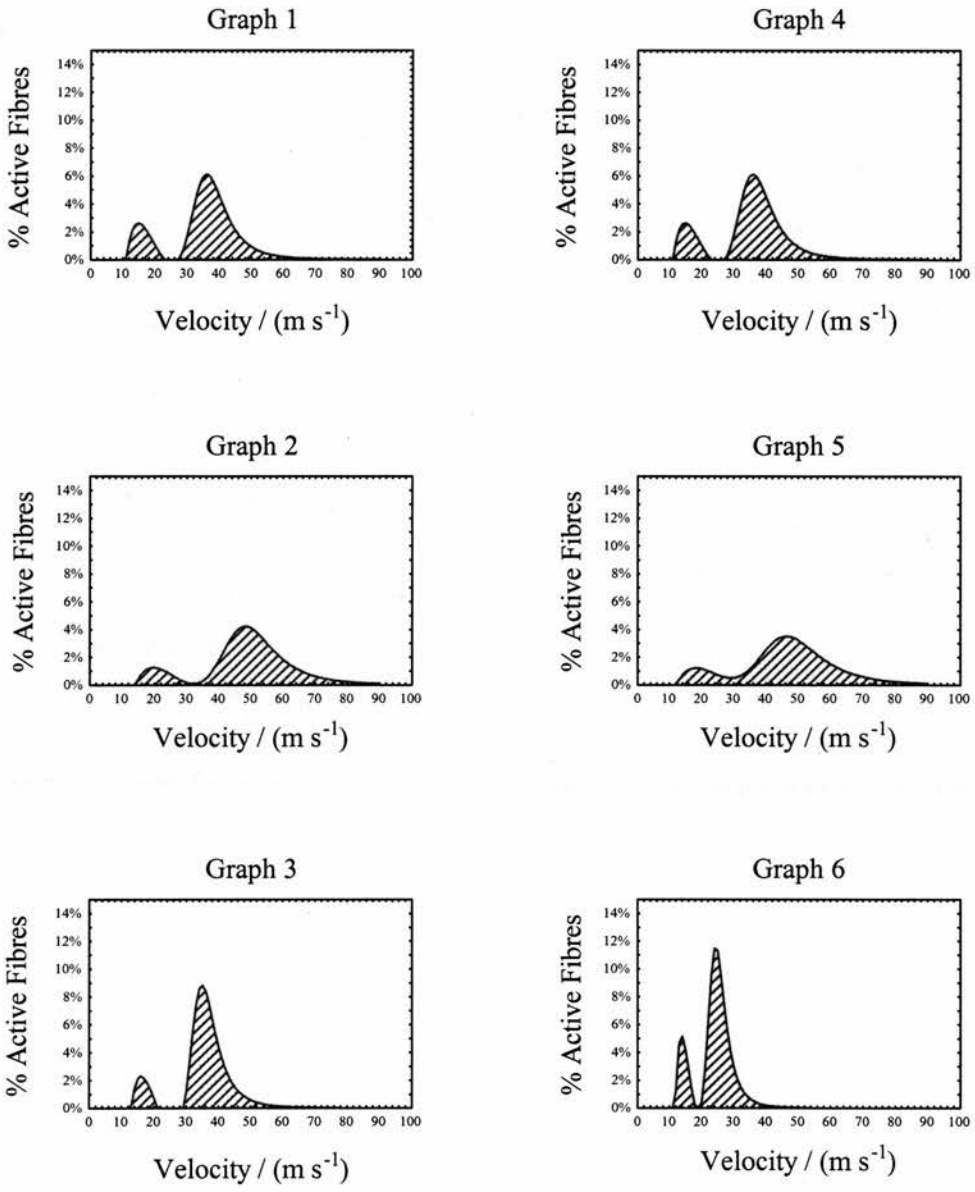


Figure 7

Facial Nerve DCV
Nerve Graft Model

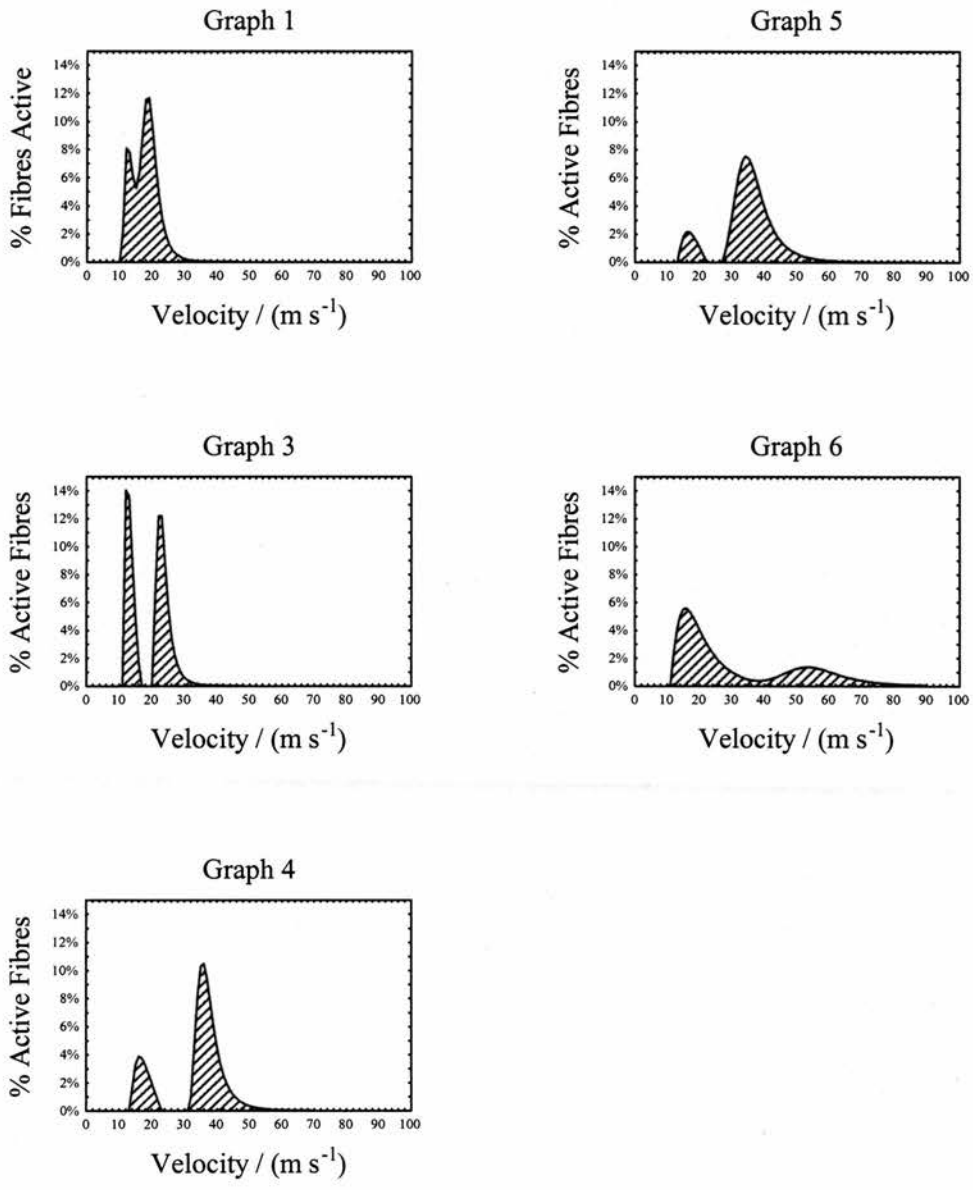


Figure 8

COMPARISON OF THE MEDIAN & FACIAL NERVES

From the results obtained in the present chapter and those obtained in Chapter 4 it was possible to compile a spreadsheet in which the columns of measured variables could be ranked both by the nerve in question and further subdivided by the method of injury and repair. Thus there were two independent variables, the first ***Nerve*** having two subgroups *Median* and *Facial* and the second subgroup ***Experiment*** having six subgroupings: *Normal, Neurapraxia, Axonotmesis, Neurotmesis, Wrap* and *Graft.*

The spreadsheet could then be used to examine the combined effects of the two independent variables ***Nerve*** and ***Expt*** upon each of the dependent variables. In choosing which of the latter to include the results obtained here and in the previous chapter were taken into account. Thus the dependent variables which had been shown to be effective discriminators in both nerves were: CV_{max}, axon diameter, fibre diameter and myelin sheath thickness. The last of these was, however, a derived function having been calculated from the axon diameter and fibre diameter and so was omitted from the ANOVA calculation. For the ***Nerve*** × ***Expt*** independent variable matrix, the Factorial ANOVA programme in Statistica showed an overall Wilks λ = 0.261 with p = 0.0000. This implied that the correlation was highly significant for at least one of the dependent variables. ANOVA in the present context is effectively a means of separating within-groups-variation from between-groups-variation and restricting the statistical test to the former. Thus the multifactorial ANOVA is equivalent to the F test described in Chapter 3. To find out where the differences lay, it was necessary to perform *post hoc* tests and as before the Scheffé test was chosen. Scheffé tests were first carried out in the Main Effects ANOVA programme to identify the overall effects. This gave the following p-values: axon diameter p = 0.599, fibre

diameter p = 0.045 and CV_{max} p = 0.019. Thus when combined effects were considered there was no significant difference to be found for axon diameter, fibre diameter was just significant and CV_{max} was reasonably significant. Given the fact that morphometric indices of outcome have no clinical use it was encouraging to find that, at the end of an exhaustive set of tests, CV_{max} had maintained its supremacy and emerged as the most discriminatory index of recovery. Taking CV_{max} as the only dependent variable a repeat of the ANOVA test gave the following results:

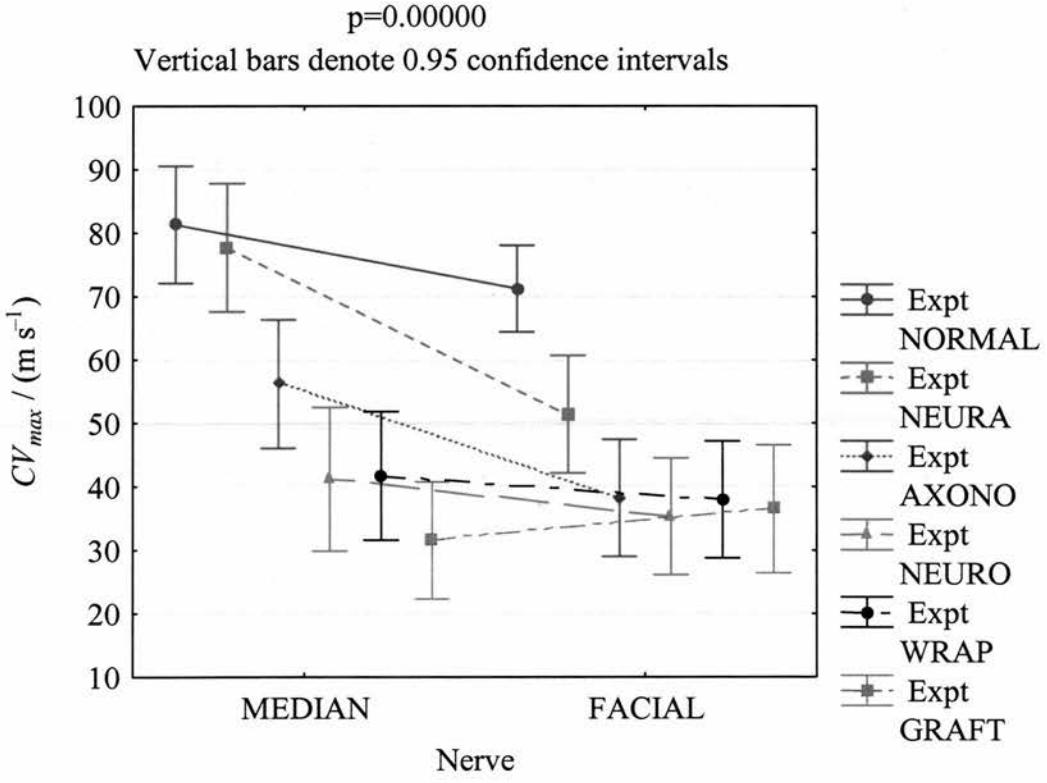


Figure 9

A graphical summary of the combined effects of Nerve and Experiment upon the value of the dependent variable CV_{max}

The precise correlations revealed by the Scheffé test for CV_{max} taking into account all combinations of the subgroups within the independent variables were (Table 3):

Cell No.	Nerve	Expt	{1} 81.383	{2} 77.800	{3} 56.260	{4} 41.275	{5} 41.680	{6} 31.533	{7} 71.309	{8} 51.533	{9} 38.283	{10} 35.433	{11} 38.000	{12} 36.540
	Scheffe test; variable CVmax (MEDIAN & FACIAL) Probabilities for Post Hoc Tests Error: Between MS = 127.75, df = 59.000													
1	MEDIAN	NORMAL		1.000000	0.291316	0.006127	0.002666	0.000009	0.987308	0.057297	0.000248	0.000063	0.000217	0.000292
2	MEDIAN	NEURA	1.000000		0.615329	0.033406	0.019114	0.000154	0.999870	0.226620	0.002877	0.000868	0.002562	0.002893
3	MEDIAN	AXONO	0.291316	0.615329		0.967996	0.959616	0.316046	0.857531	0.999998	0.798325	0.599887	0.781024	0.740844
4	MEDIAN	NEURO	0.006127	0.033406	0.967996		1.000000	0.998853	0.060203	0.998154	1.000000	0.999993	1.000000	0.999999
5	MEDIAN	WRAP	0.002666	0.019114	0.959616	1.000000		0.997022	0.030248	0.997712	1.000000	0.999972	1.000000	0.999998
6	MEDIAN	GRAFT	0.000009	0.000154	0.316046	0.998853	0.997022		0.000089	0.588502	0.999902	1.000000	0.999936	0.999997
7	FACIAL	NORMAL	0.987308	0.999870	0.857531	0.060203	0.030248	0.000089		0.392299	0.003013	0.000719	0.002624	0.003500
8	FACIAL	NEURA	0.057297	0.226620	0.999998	0.998154	0.997712	0.588502	0.392299		0.960919	0.858002	0.954445	0.933197
9	FACIAL	AXONO	0.000248	0.002877	0.798325	1.000000	1.000000	0.999902	0.003013	0.960919		1.000000	1.000000	1.000000
10	FACIAL	NEURO	0.000063	0.000868	0.599887	0.999993	0.999972	1.000000	0.000719	0.858002	1.000000		1.000000	1.000000
11	FACIAL	WRAP	0.000217	0.002562	0.781024	1.000000	1.000000	0.999936	0.002624	0.954445	1.000000	1.000000		1.000000
12	FACIAL	GRAFT	0.000292	0.002893	0.740844	0.999999	0.999998	0.999997	0.003500	0.933197	1.000000	1.000000	1.000000	

Table 3

A matrix in which the combinations of nerve and experiment are listed vertically on the left and are represented by numbers horizontally. The numbers in red are the probability values indicating significant differences.

CONCLUSIONS

From a consideration of the experiments presented in this chapter it appears that by applying the CV_{max} test it is possible to discover differences in CV_{max} in a large number of the subgroups seen in Table 3. It is important to bear in mind also that the distribution of fibre diameters in the buccal branch of the facial nerve would have been shifted to the left of that seen in the median nerve as it is a smaller nerve trunk. In all cases except that of the nerve autograft the mean velocity in the facial nerve was a few m s^{-1} slower than in the median nerve. In the human where there are many large-diameter motor and proprioceptive fibres in the median nerve, this difference would be more marked.

From a clinical point of view it is unlikely that these small differences in velocity would show up as any clinically measurable effect or as any effect which the patient might notice. Events taking place when the unimodal nerve regenerates may be thought to be favoured by the reduced randomness of the process by which the pioneering axons reach their targets — i.e. all targets will be either motor or sensory but never a mixture. However any preferment which this might be supposed to bring must be considered not proven in the light of the evidence presented here.

CHAPTER 6 APPLICATION OF CV_{DIST} TO THE HUMAN SUBJECT

WHILE the general theme of the present work is that of evaluation of the sheep model and of specific models therein of nerve injury and repair, the emergence of CV_{Dist} as a test of considerable diagnostic merit was thought to be worthy of inclusion. Consequently a number of preliminary experiments were carried out on normal volunteers as an assessment of the test in a clinical setting. Given that the work presented in Chapter 4 pointed to the value of CV_{Dist} as a sensitive test offering more information than CV_{max} , the objectives of this secondary study were of a more practical nature. Specifically:

1. To examine the tolerance by the human subject of the repetitive stimulation
2. To determine the nature and cost of the necessary consumable equipment.
3. To determine the time taken to complete a test
4. To determine the test's applicability to a range of commonly injured nerves.
5. To examine the test in generalized afflictions of nerve e.g. ischaemia.
6. To examine the variability/reproducibility of the results in human subjects.

CV_{DIST} IN THE HUMAN SUBJECT

Volunteers for this group of experiments were recruited from the staff working at the Department Of Clinical Neurosciences, Western General Hospital, Edinburgh.

Sixteen normal volunteers took part in this study. None of the volunteers had a past medical history of neurological disease or diabetes. A detailed explanation of the procedure was given and informed consent was obtained. Volunteers were allowed to

rest comfortably in a chair with their upper limbs supported on pillows and in a warm environment. Their upper limbs were exposed to above the elbow and allowed to equilibrate to room temperature prior to commencing the examination.

Stimulating electrodes were placed over the anatomical surface landmarks for the median nerve at the elbow and wrist (Delisa et al. 1994). Two disposable adhesive nerve stimulating pads were used at each site (Oxford Instruments, U.K.). Recording electrodes of a similar type were placed on the hand, with the active electrode over the motor point of abductor pollicis brevis and with a reference electrode placed on the index finger. A ground electrode was placed on the palm between the recording and stimulating electrodes.

The baseline surface temperature was recorded from the patient's forearm just proximal to the distal electrode with a laser-guided infrared thermometer (Raytex U.K. Ltd.), The distance between the proximal and distal electrodes was measured to the nearest millimetre.

In order to estimate the distribution of motor conduction velocities it was necessary to record both CV_{max} and the refractory period. Initially maximum motor conduction velocity (CV_{max}) was recorded. This was calculated in the usual way from the difference in M-wave latencies after stimulating at the two sites.

The absolute refractory period was recorded using a paired shock technique (McDowall K.L. et al. 1998). Supramaximal stimulation was applied to the median nerve from the distal stimulating electrode and the resulting M-wave recorded as a reference trace. Incremental time delays were introduced until a change was detected in the M-wave. This represented the start of a second M-wave. In practice, the second M-wave was detected by digitally subtracting the reference trace from the active trace. The time delay at which the second M-wave appeared was called the absolute

refractory period and was assumed to be the absolute refractory period of the fastest fibres. The relative refractory period could be estimated as described in Chapter 3 and was taken to be the absolute refractory period of the slowest fibres.

The volunteers were given a list of thirty pre-determined inter-stimulus intervals and were requested to call-out the numbers to the examiner. Involving the volunteers with this simple task, helped to distract them from any discomfort associated with the stimulation.

The stimulus required to produce a maximal CMAP was established initially at the two stimulation sites. The stimulus intensity was set at 1.5 times the minimum amplitude that produced a full CMAP. The subsequent M-waves elicited after stimulating at each of the 30 inter-stimulus intervals were recorded and stored in consecutive channels in the EMG machine. The CV_{Dist} profile was calculated as outlined in chapter 3.

APPLICATION OF THE CV_{DIST} TEST IN THE HUMAN

Motor CV_{Dist}

A typical distribution profile of the motor conduction velocities within a normal human median nerve is shown in Figure 1. It was found that the distribution had a characteristic bi-modal shape, with a group of slow conducting fibres and a larger group of faster conducting fibres. Although there was variation among individuals with regard to the size and speed of these two groups, the bimodal distribution was evident in all volunteers.

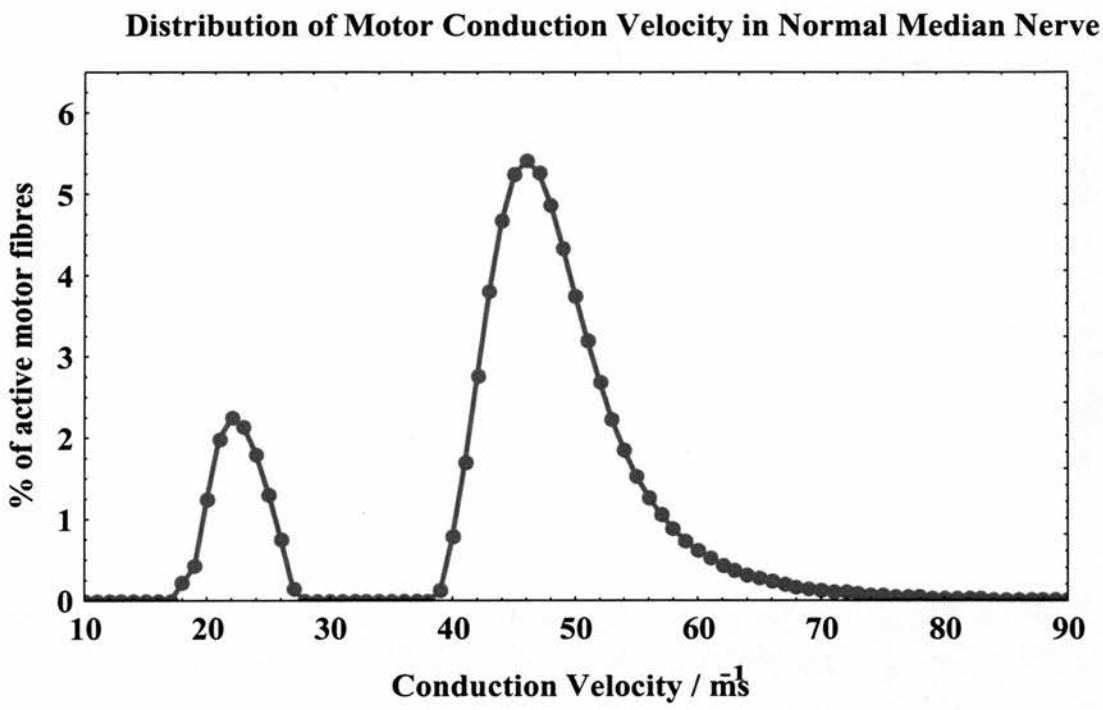


Figure 1

A typical distribution of motor conduction velocities in the median nerve[19].

The average distribution of the male and female volunteers is shown in Figure 2. Both males and females had similar distribution profiles. Bilateral studies of the median nerve were performed and no difference was found between the dominant and non-dominant sides (Figure 3).

[19] The software was recalcitrant with the units on the x-axis which in Figures 1– 20 should read: **Conduction velocity / (m s^{-1}).**

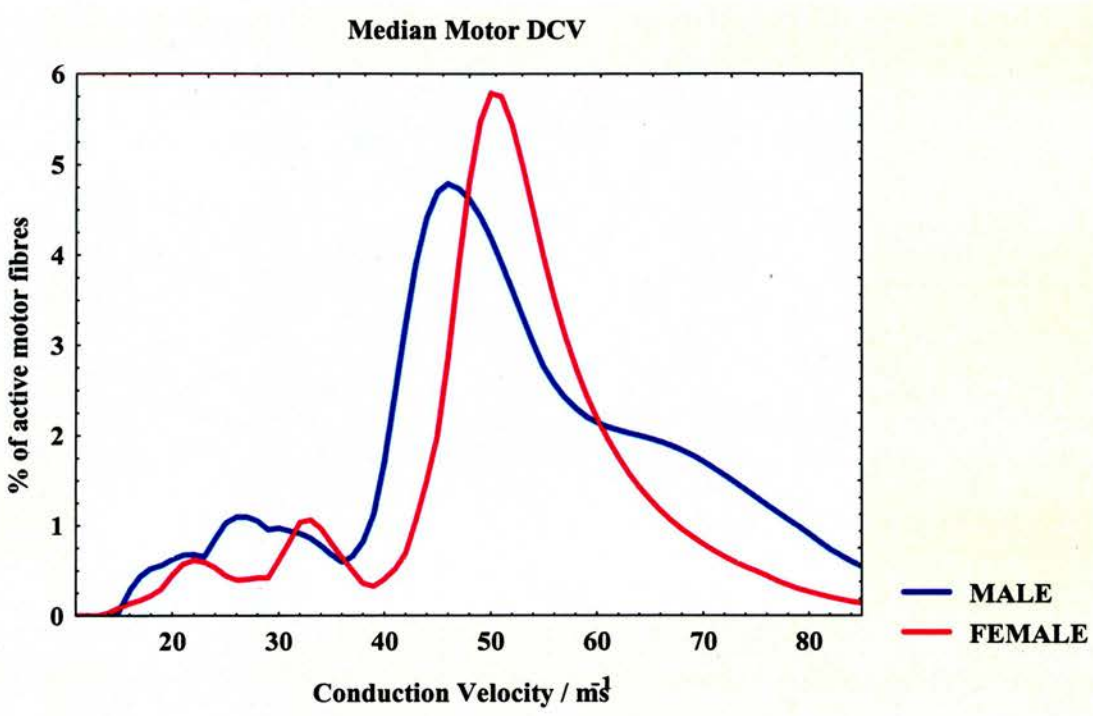


Figure 2

Graph showing the average motor CV_{Dist} distribution for the male (n=7) and female (n=7) volunteers.

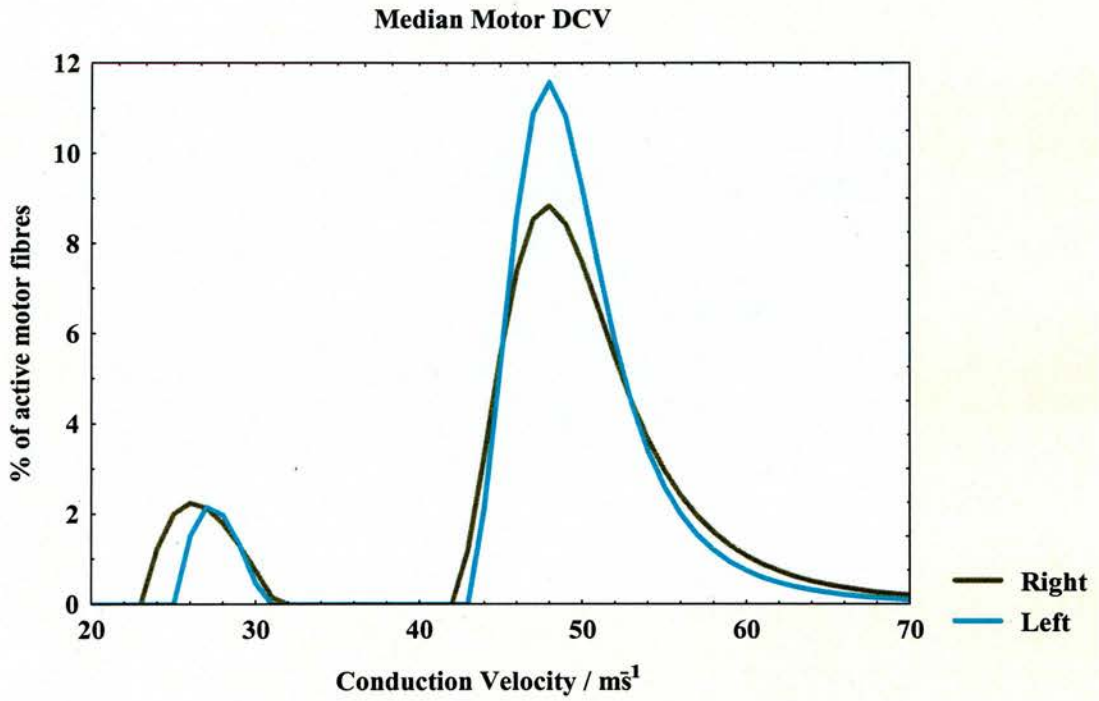


Figure 3

Comparison of dominant and non-dominant sides

Surface forearm temperature readings were between 32°C to 35°C in all volunteers. No adjustment was made for temperature in this range.

To assess reliability four sequential recordings were carried out on the volunteers over a period of six weeks. Examples of four typical recordings are shown in Figure 4.

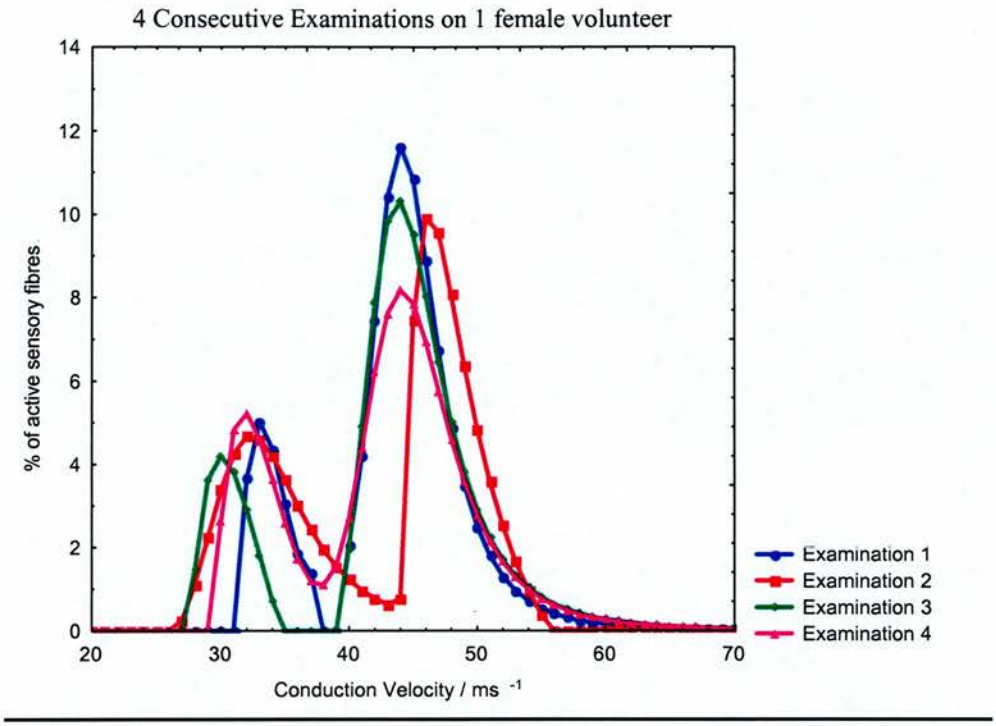


Figure 4

Four consecutive DCV recordings over six weeks in one female volunteer.

The distribution profiles were consistent over the four examinations.

The correspondence between CV_{max} and CV_{Dist} was also investigated. In Figure 5, the distribution profiles of four of the volunteers have been shown each with an arrow pointing to his corresponding maximum conduction velocity.

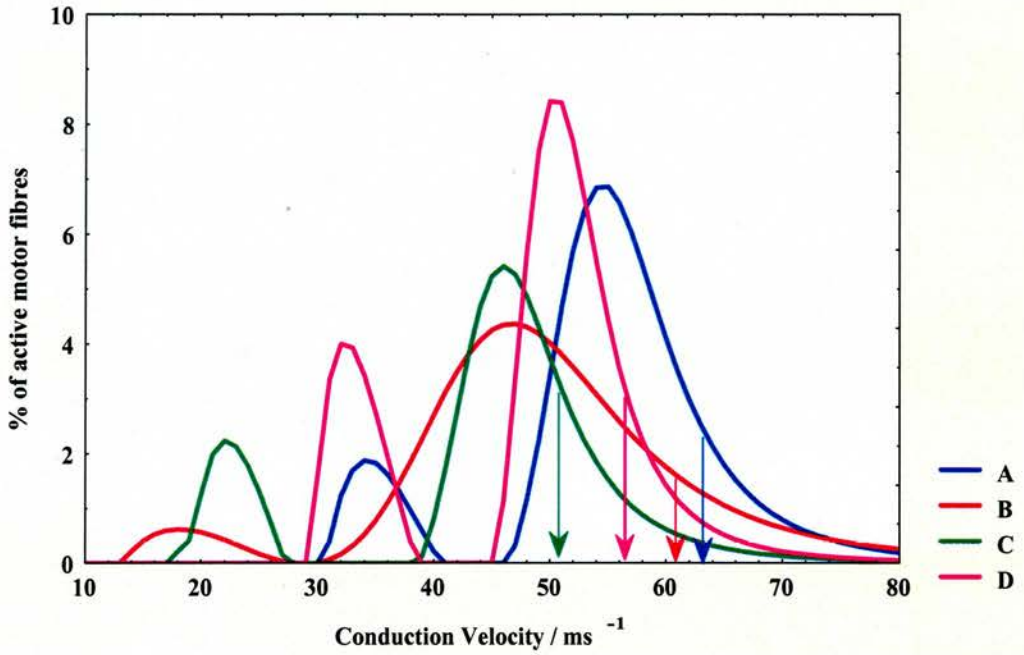


Figure 5

Distribution profiles of four volunteers (A – D) with an arrow pointing to their maximum conduction velocities.

With practice the average length of time taken to set-up and record motor CV_{Dist}, maximum conduction velocity and refractory period in the volunteers was fifteen minutes. A further ten minutes was needed to calculate the area of the M2 waves and to process the data.

Examples of Motor CV_{Dist} in other nerves

Motor CV_{Dist} recordings were also obtained from the ulnar and common peroneal nerves. The purpose of these recordings was for comparison with the median nerve recording to see if the bimodal distribution was characteristic of other motor nerves and if this method of recording CV_{Dist} was sensitive enough to distinguish between different motor nerves. The same principles and methods were used in the recording of motor CV_{Dist} in the ulnar and common peroneal nerve.

Ulnar CV_{Dist}

Standard stimulation points were chosen at the elbow and just proximal to the wrist as outlined by Delisa (Delisa, Lee, Baran, Lai, Spielholz, & MacKenzie 1994). Recording electrodes were placed over the hypothenar eminence. A ground electrode was placed between the recording and stimulating electrodes.

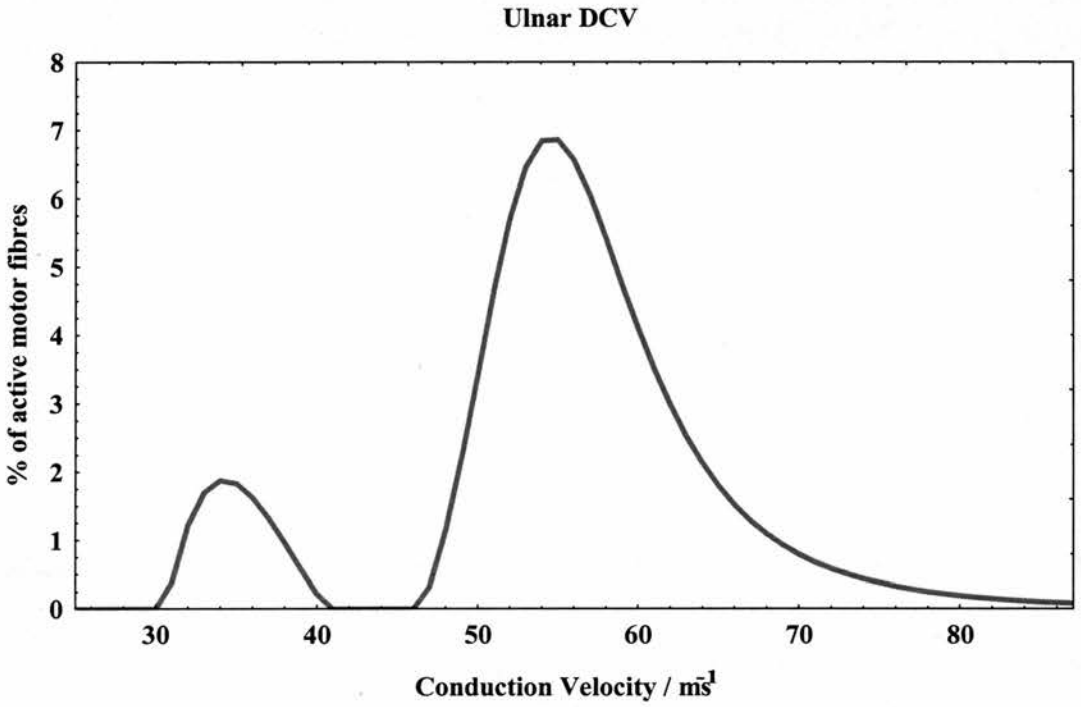


Figure 6

Example of ulnar nerve motor CV_{Dist}

There was little difference to be found in the general appearance of the CV_{Dist} curve for the ulnar nerve when compared with that of the median nerve (Figure 6).

Likewise when male and female volunteers were compared there was found to be no appreciable difference in the shape and size of the CV_{Dist}.

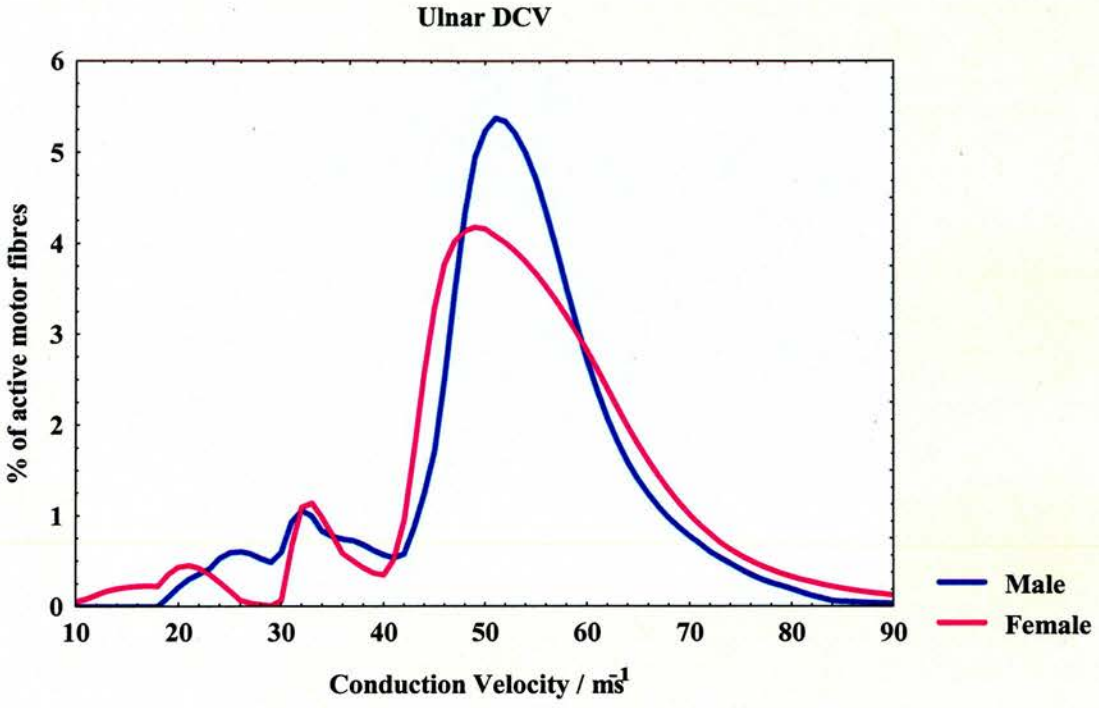


Figure 7

Average male (n=6) and female (n=7) ulnar motor CV_{Dist}

Common Peroneal CV_{Dist}

Standard neurophysiological stimulation sites were chosen above and below the knee (Delisa, Lee, Baran, Lai, Spielholz, & MacKenzie 1994). Recording electrodes were placed over extensor digitorum brevis.

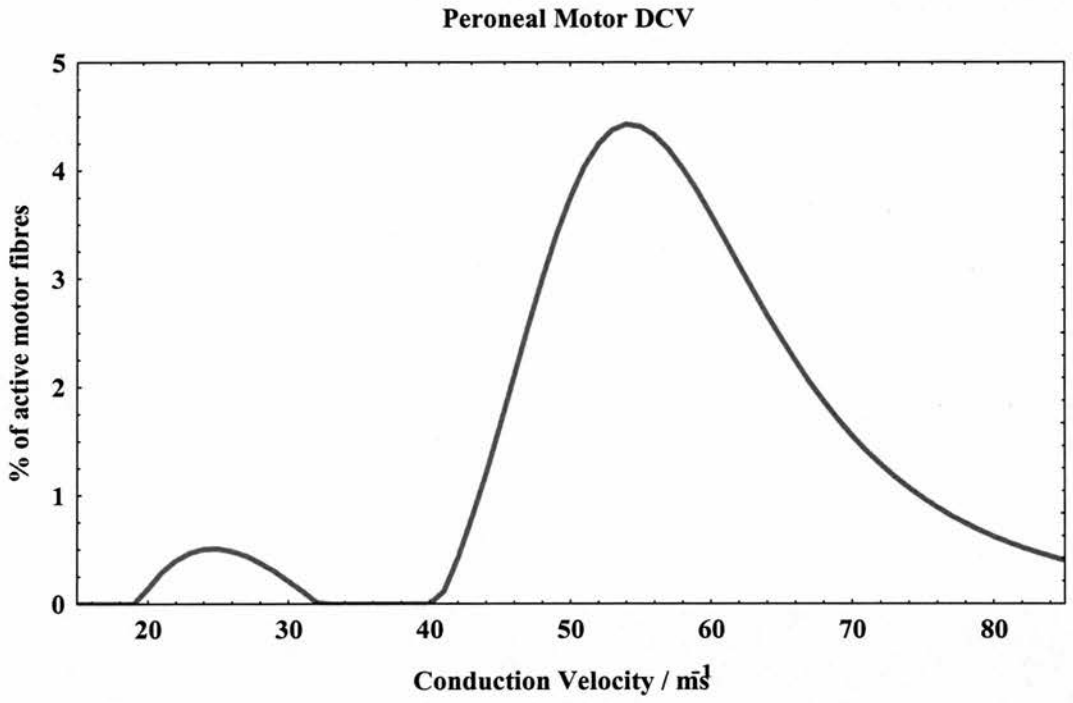


Figure 8

Common peroneal motor CV_{Dist} in a single female volunteer

Comparison of motor CV_{Dist} in 3 different nerves

All three nerves were found to have a similar motor CV_{Dist} profile with a typical bimodal distribution. The accepted normal maximum conduction velocity range for the median, ulnar and common peroneal nerves are as follows (Kimura 2001).

Nerve	**CV_{max} (m s^{-1})**
Median (elbow)	57.7 ± 4.9
Ulnar (elbow)	61.0 ± 5.5
Common Peroneal (knee)	52.0 ± 6.2

Table 1

Kimura's values for CV_{max}

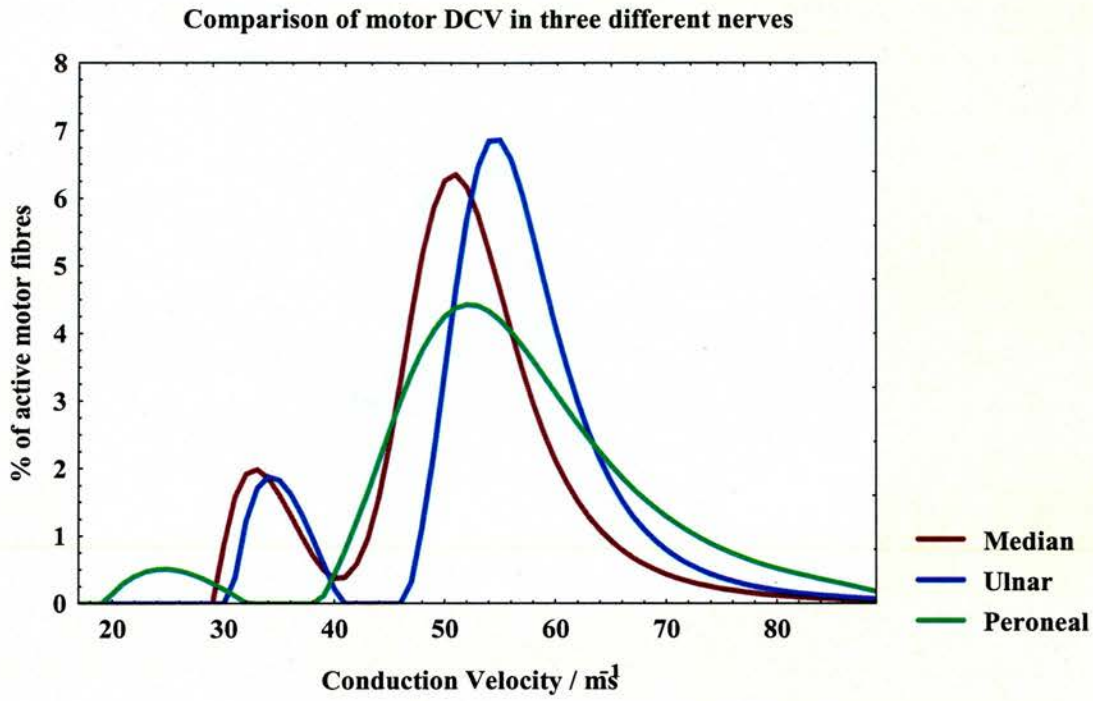


Figure 9

Experimentally determined CV_{Dist} and CV_{max} for median, ulnar and common peroneal nerves.

Sensory CV_{Dist}

Estimation of sensory CV_{Dist} would probably be of even greater clinical benefit than motor CV_{Dist} in the diagnosis and management of various neuropathies. It is not easy to measure sensory CV_{Dist} in the sheep because of the difficulty in isolating adequate peripheral nerves which are known to be wholly sensory. In the human, however, the technique is essentially the same as that for motor CV_{Dist}. That is not however, to say that the data can be fitted to a curve with the same equation and further work is necessary to elucidate this. For the present experiments the same formula and similar methods were employed and it must be appreciated that by making such an assumption about the fitted equation, this can be no more than an exploratory study.

Methods

Subjects were recruited from the same group of volunteers as the motor CV_{Dist} study. The median nerve on the left side was chosen for study. The study was carried out antidromically as better recordings can be elicited from the digital nerves as they are more superficially situated and are purely sensory. Recording ring electrodes were placed around the middle finger; the anode over the distal phalanx and the cathode over the proximal phalanx. Stimulating and ground electrodes were placed at similar locations to these used in the motor recording. The volunteer's hand was strapped securely to an arm board to minimize interference from movement. The sensitivity of the recording amplifier had to be greatly increased owing to the much lower amplitudes of the sensory action potentials. It is this which makes the recording of sensory CV_{Dist} much more difficult, as the signal-to-noise ratio is much reduced. As a result of the increased sensitivity there was an increased amount of noise artefact, making the measurement of the latency of the CSNAP more difficult. In practice the sweep speed was set at 20 ms cm^{-1} and the stimulation rate was set at 1 Hz. The current was increased until a compound sensory action potential (CSNAP) could be detected on the screen at a sensitivity of 10 or 20 μV per division. Again all stimulations were supramaximal (1.5 times the minimum current amplitude required to produce a full CNAP).

Results

A typical distribution profile of the sensory conduction velocities within a normal median nerve is shown in Figure 10. Similar to the motor CV_{Dist} a bimodal distribution was evident. There was greater variation among individuals with regard to the size

and speed of these two groups. Both males and females were found to have similar distribution profiles.

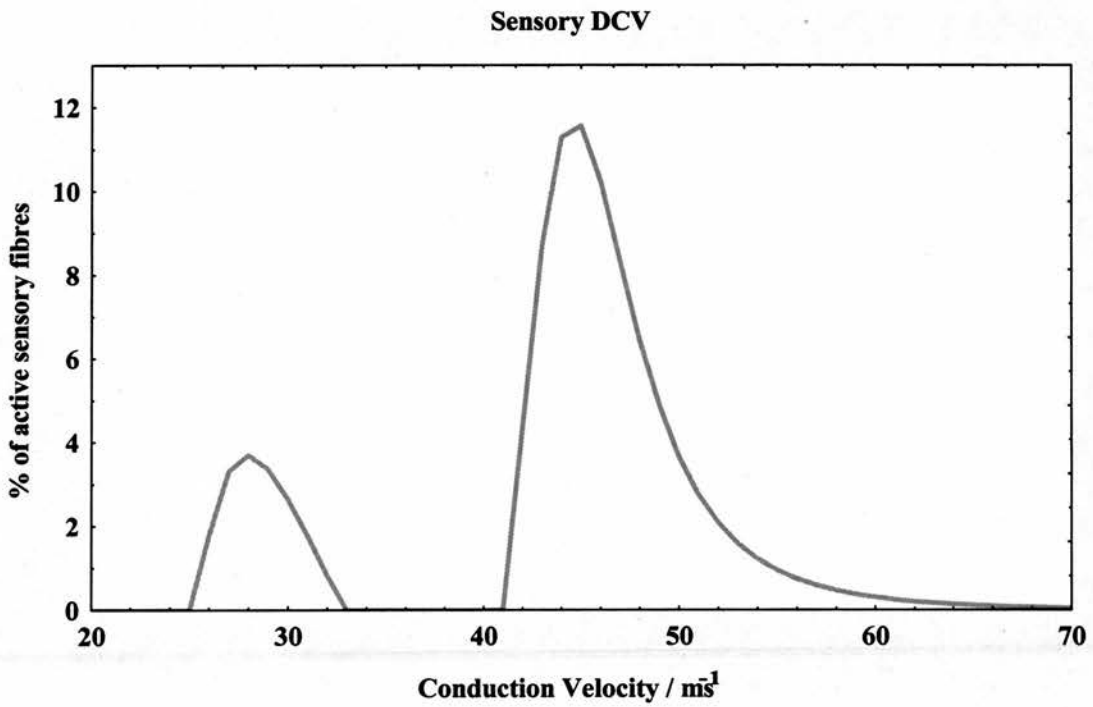


Figure 10

A typical distribution of sensory conduction velocities in the median nerve.

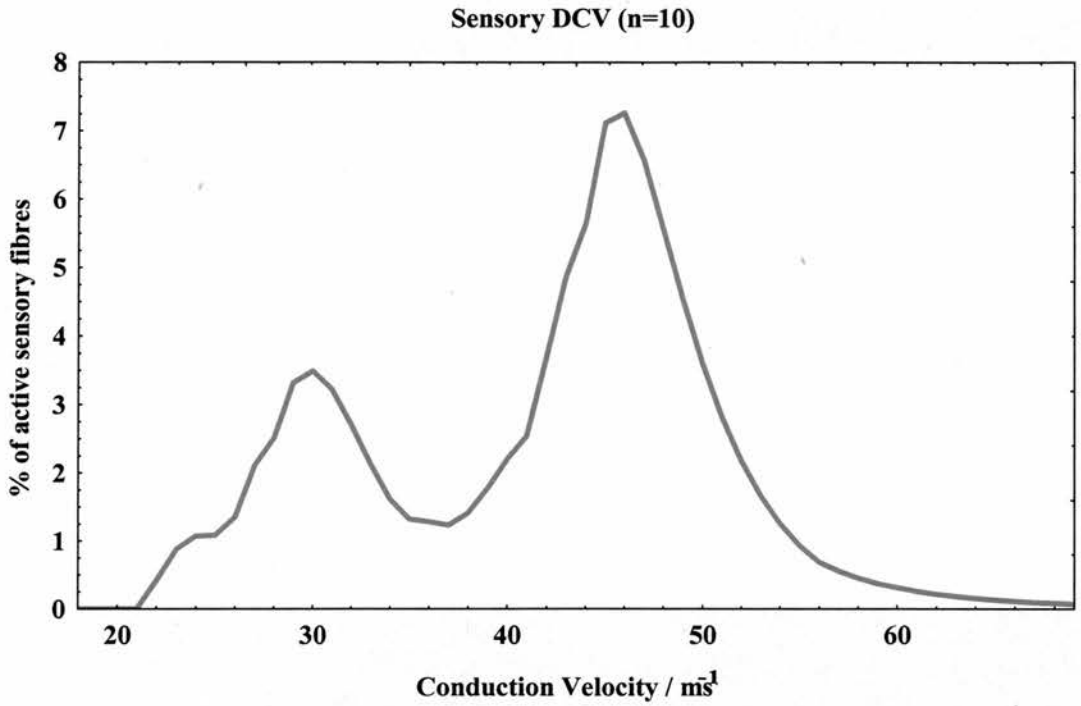


Figure 11

Graph showing the average distribution of sensory CV_{Dist} in all of the volunteers.

Bilateral studies of the median nerve were performed and no difference was found between the dominant and non-dominant sides (Figure 12). Surface forearm temperature readings were between 32°C to 35°C in all of the volunteers. No adjustment was made for temperature in this range. To assess reliability four sequential recordings on the volunteers were carried out over a period of six weeks. Examples of four typical recordings are shown in Figure 13–15. The distribution profiles were consistent over the four examinations.

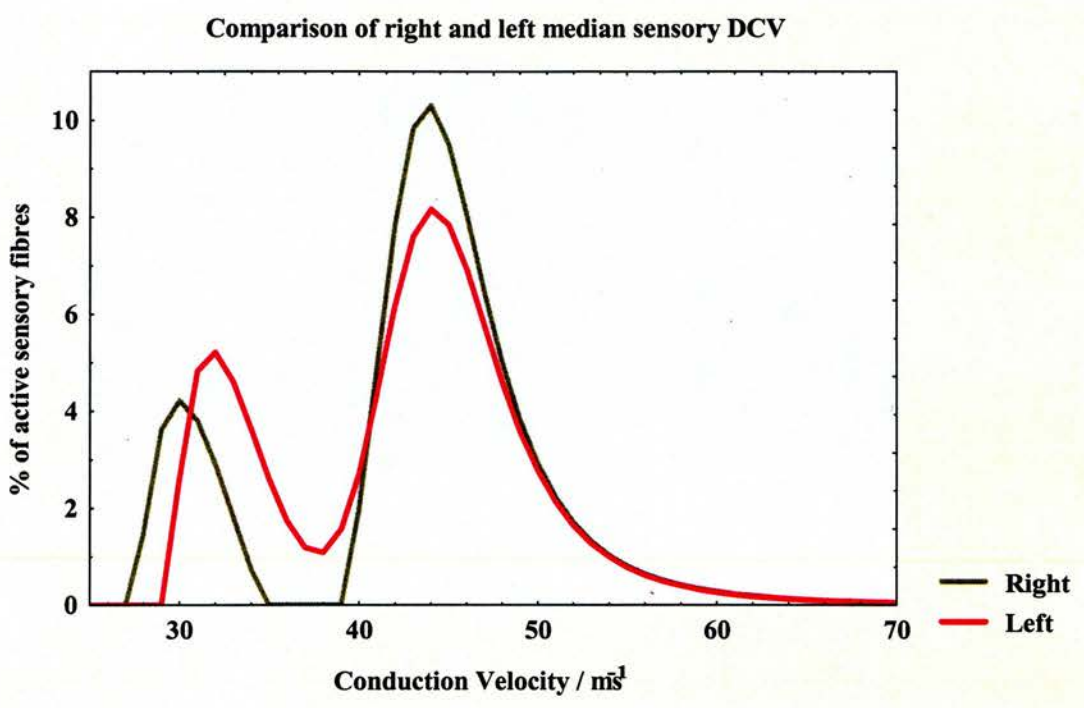


Figure 12

Comparison of sensory CV_{Dist} in dominant and non-dominant sides

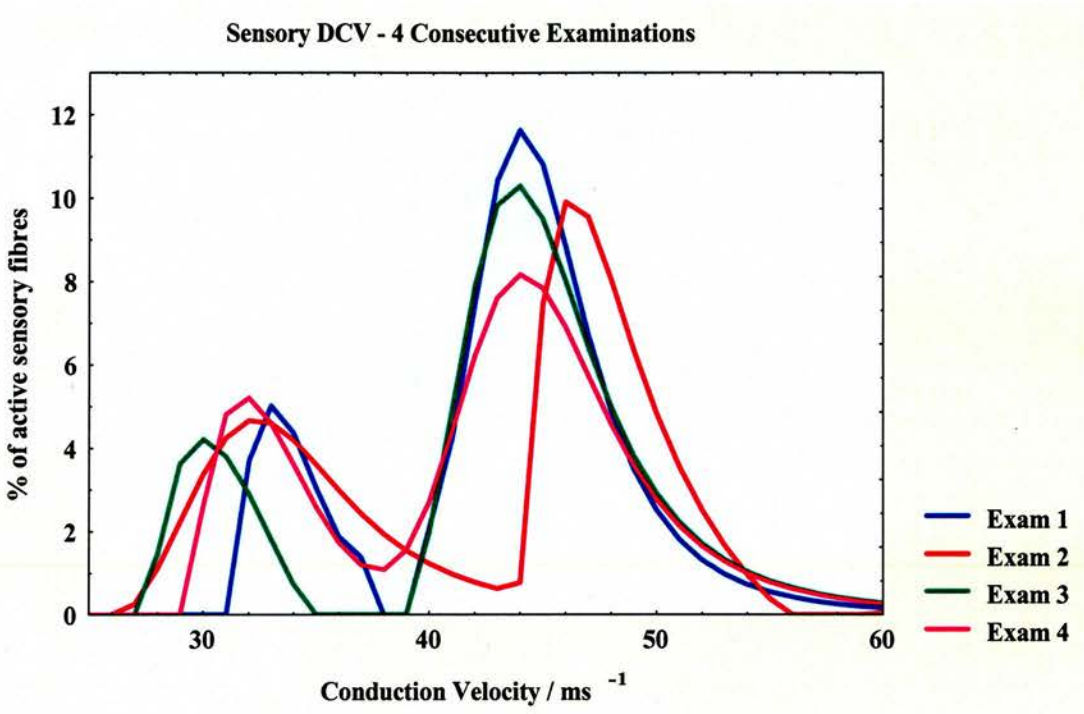


Figure 13

Four consecutive sensory CV_{Dist} recordings over six weeks in one volunteer.

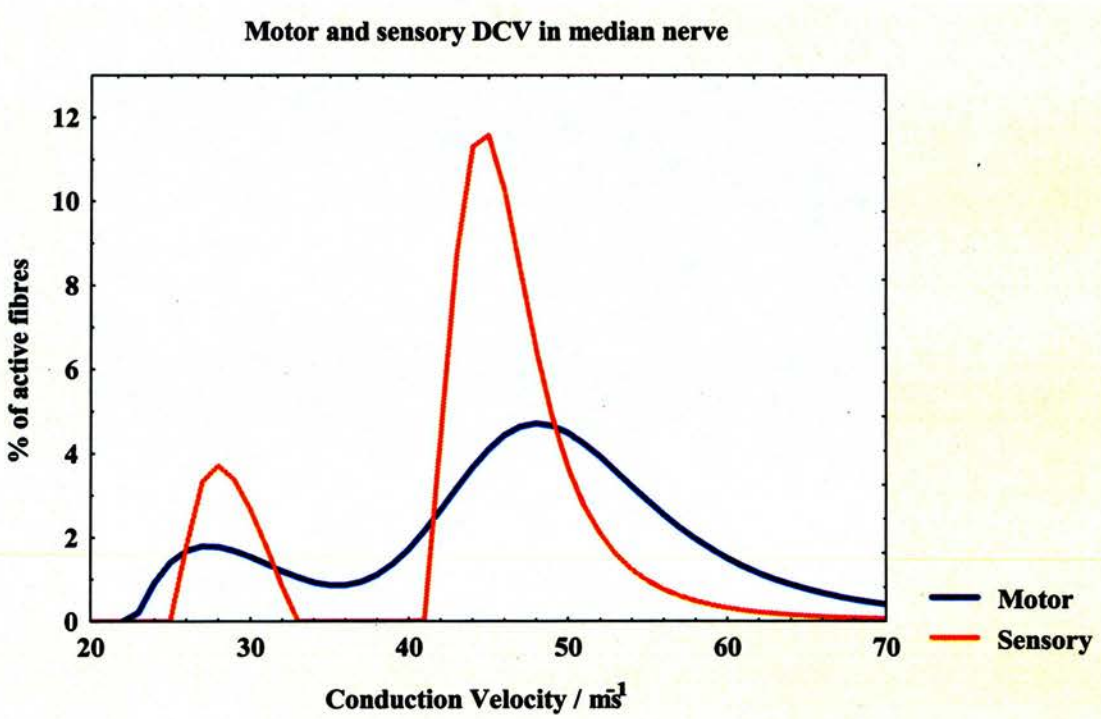


Figure 14

A comparison of the profiles of sensory and motor CV_{Dist} in the median nerve of a single volunteer.

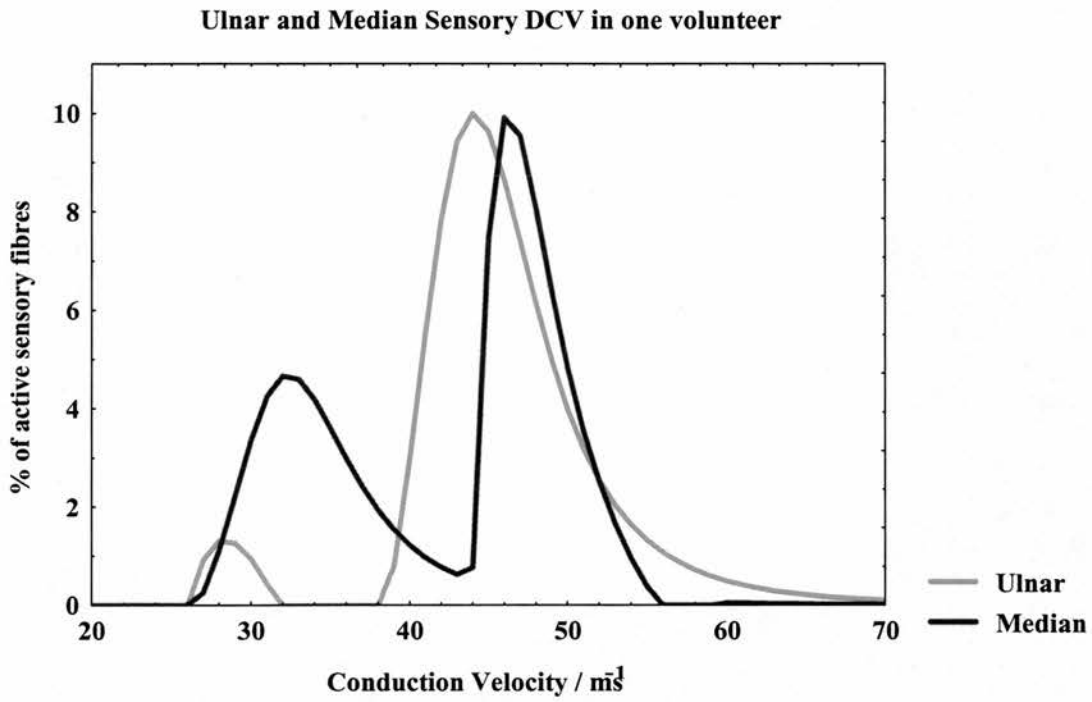


Figure 15

Comparison of median and ulnar sensory CV_{Dist} in a single volunteer

The average length of time taken to set-up and record sensory CV_{Dist} was 15 minutes. A further ten minutes was needed to calculate the area of the CSNAP waves and to process the data.

Temperature

Lower temperatures slow down the propagation of impulses. For example, distal latencies increase by 0.3ms degree^{-1} for both median and ulnar nerves upon cooling of the hand (Kimura 2001). Cold-induced slowing of sodium channel opening and a delay in the activation of g_{Na}^{+} probably account for the slowing of conduction. A parallel temperature-dependent change also affects the refractory period (Kimura 2001).

Because cold is known to have a discernable effect on conduction velocity, its effect on CV_{Dist} was measured in 2 volunteers. Median nerve temperature was lowered by a combination of exposing the upper limb and by immersing it in ice-cold water. The surface temperature was lowered below the test temperature and then allowed to rise slowly. Motor CV_{Dist} recordings were obtained at a distal surface temperature of 27°C.

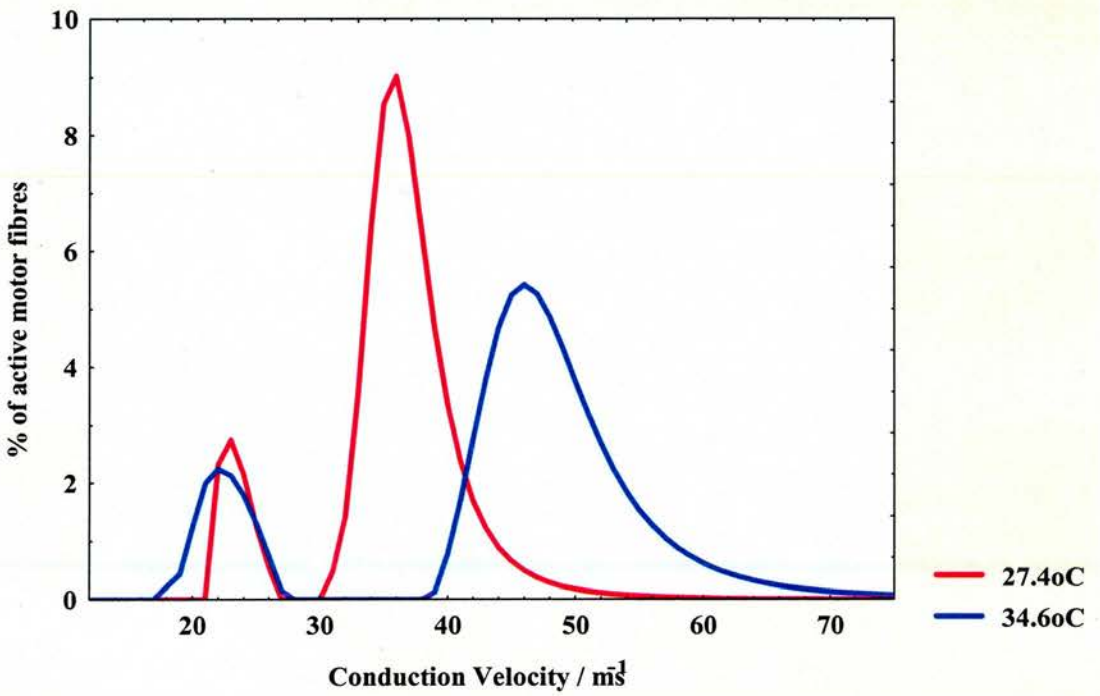


Figure 16

Comparison of median nerve sensory CV_{Dist} before and after cooling. Temperatures are in °Celsius.

From this it can be seen that cooling has a greater effect upon the faster fibres. This is a well-documented effect.

Ischaemia

It is generally accepted that peripheral nerves have a great resistance to injury caused by ischaemia and are capable of surviving for several hours without a blood supply (Abramson et al. 1971). Periods of tourniquet-induced ischaemia cause slowing of

conduction velocity in nerve fibres underneath the tourniquet and distal to the occlusion (Abramson, et al 1971; Mogyoros et al. 1997). The mechanism for this is not fully understood but it has been attributed to the effects of mechanical blockage of the arterial supply (Rorabeck 1980) inhibition of the electrogenic Na^+/K^+ pump (resulting in an extracellular accumulation of K^+ ions and consequent depolarization of the membrane), and the metabolic effects of ischaemia (*op cit*).

Nathan described the effects of experimental ischaemia induced with a tourniquet as having an early phase of tingling paraesthesiae lasting from about the third to the sixth minute of arterial occlusion (Nathan 1958). Normal sensation then recovered till the onset of anaesthesia and paralysis, usually at about 25 minutes in the arm. On release of the tourniquet he found that there was an immediate but brief period of normal sensation, followed by several minutes of severe pain and paraesthesiae. During the early phase of painless paraesthesiae the sensation of touch was retained but the sensation of pain was greatly diminished or lost. In the stage of continuous pain and severe paraesthesiae that followed removal of the tourniquet, all forms of peripheral sensitivity were lost.

Some pathological states affect the sensitivity a nerve has for ischaemia. Gilliatt et al. found that patients with carpal tunnel syndrome were more sensitive to the effects of ischaemia whilst patients with diabetic or uraemic polyneuropathy showed a greater resistance to ischaemia than normal subjects (Gilliatt & Wilson 1954).

The restoration of maximum conduction velocity occurs within minutes after the circulation is restored (Fullarton 1995). There remains, however, a latent effect for some time, as judged by the increased responsiveness to a second period of arterial occlusion.

The aim of the present experiments was to investigate what effect a given period of ischaemia would have on both motor and sensory CV_{Dist} and to see if the clinical symptoms described by Nathan correlated to changes in the CV_{Dist} profile. Finally, to see if the recovery of conventional maximum conduction velocity heralded a return to normality for all of the fibres.

Methods

A baseline motor and sensory CV_{Dist} was recorded from the subject prior to induction of ischaemia and the surface temperature of the forearm obtained. The subject's blood pressure was recorded by means of a sphygmomanometer cuff around the subject's upper arm. This was then inflated to 210 mm Hg and maintained in the range 200-220 mm Hg (i.e. 80-100 mmHg above systolic blood pressure) throughout the course of the experiment. At various time intervals, CV_{Dist} and temperature readings were recorded. The subject was asked to describe the changing sensations in the upper limb and his observations were noted. After 20 minutes of ischaemia, the cuff was deflated. CV_{Dist} and temperature were again recorded at various time intervals for up to 60 minutes after the removal of the cuff. The experiment was repeated three times; once each to record motor and sensory DCV and once with the subject being asked to perform the nine-hole peg test, in the hope that this would give some index of changing fine motor function. The nine hole peg test has been well validated in a wide range of neurological disease processes as a good measure of upper limb function (Mathiowetz, Weber, & Kashman 1985; Worthington & DeSouza 1989).

Results

Within two minutes of arterial occlusion the motor CV_{Dist} histogram was seen to be shifted to the left as the ischaemia progressively worsened until finally motor CV_{Dist}

could no longer be recorded. This occurred at 20 minutes and coincided with the onset of paralysis and anaesthesia. After release of the tourniquet the motor CV_{Dist} gradually recovered. A characteristic, but slower, CV_{Dist} profile was evident within 2 minutes of reperfusion however the motor CV_{Dist} profile did not return to baseline until 15 minutes after the occlusion was discontinued. There was evidence of 'overshoot' on the motor CV_{Dist} recording taken at 45 minutes and this returned to baseline by 60 minutes. With the nine-hole peg test, notwithstanding evidence of a progressive leftward-shift of the motor CV_{Dist}, fine motor function did not deteriorate until after 15 minutes of ischaemia. Paralysis ensued at around the twenty minutes. Function was restored rapidly after releasing the tourniquet and baseline nine-hole peg-times were achieved 5 minutes after reperfusion began. Again this was despite continuing evidence of subnormal velocities. The surface temperature dropped nearly 2°C during the ischaemic period and gradually increased again with reperfusion. No adjustment for temperature was made since it did not drop below 32.8°C.

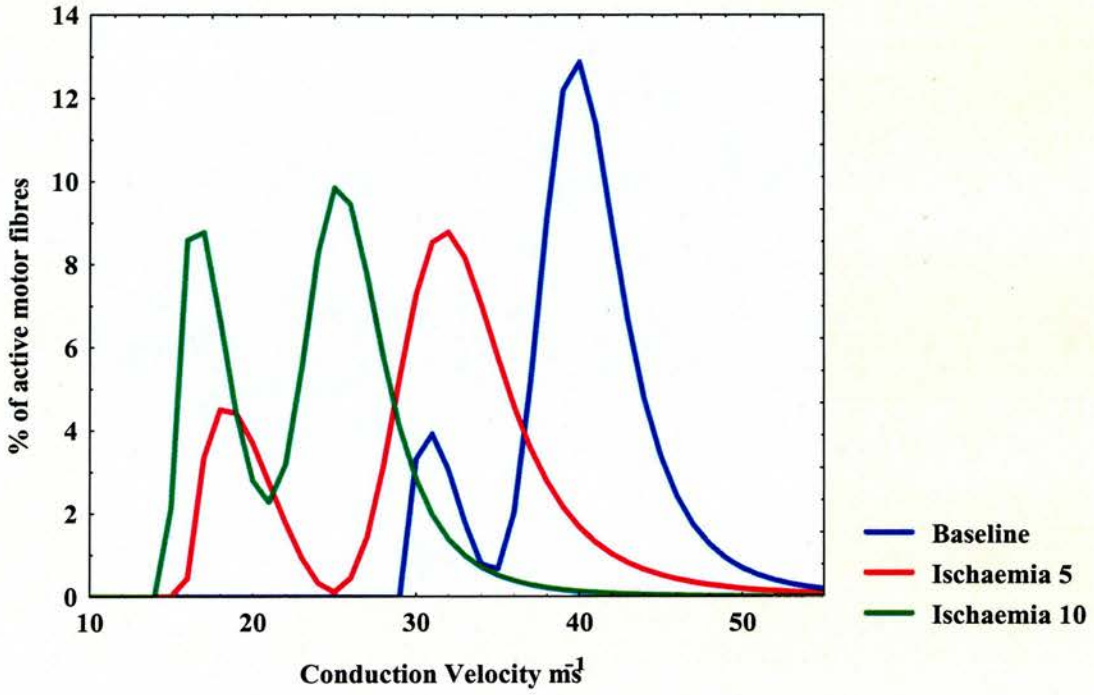


Figure 17

Motor CV_{Dist} during induction of ischaemia 0–10 minutes

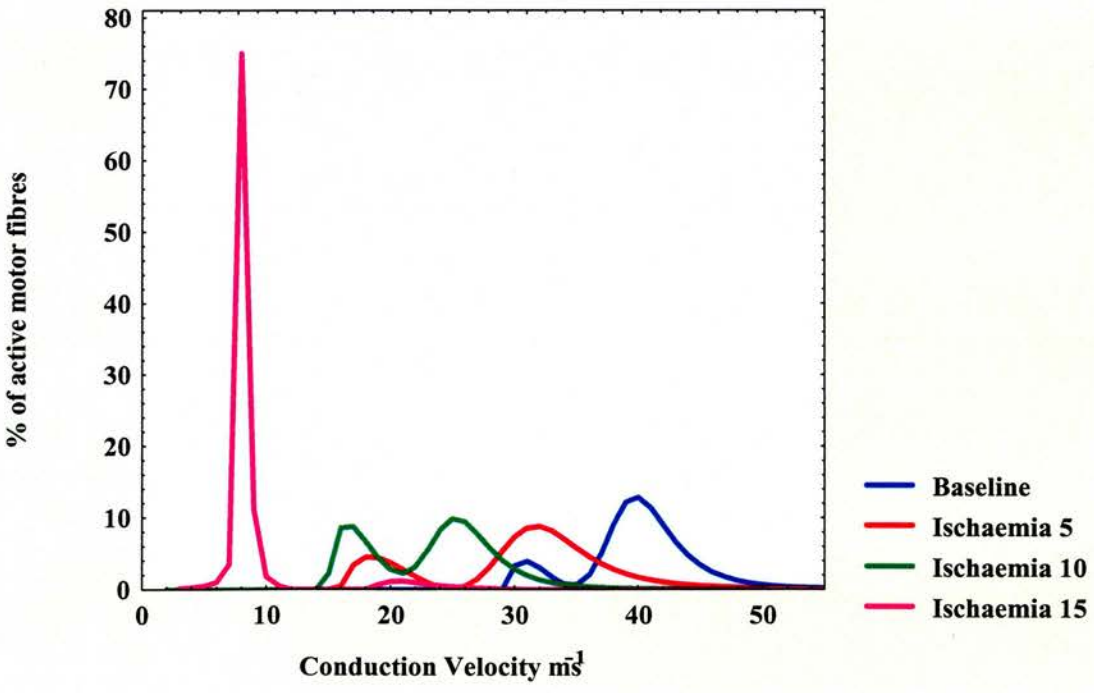


Figure 18

Motor CV_{Dist} during induction of ischaemia 0–15 minutes

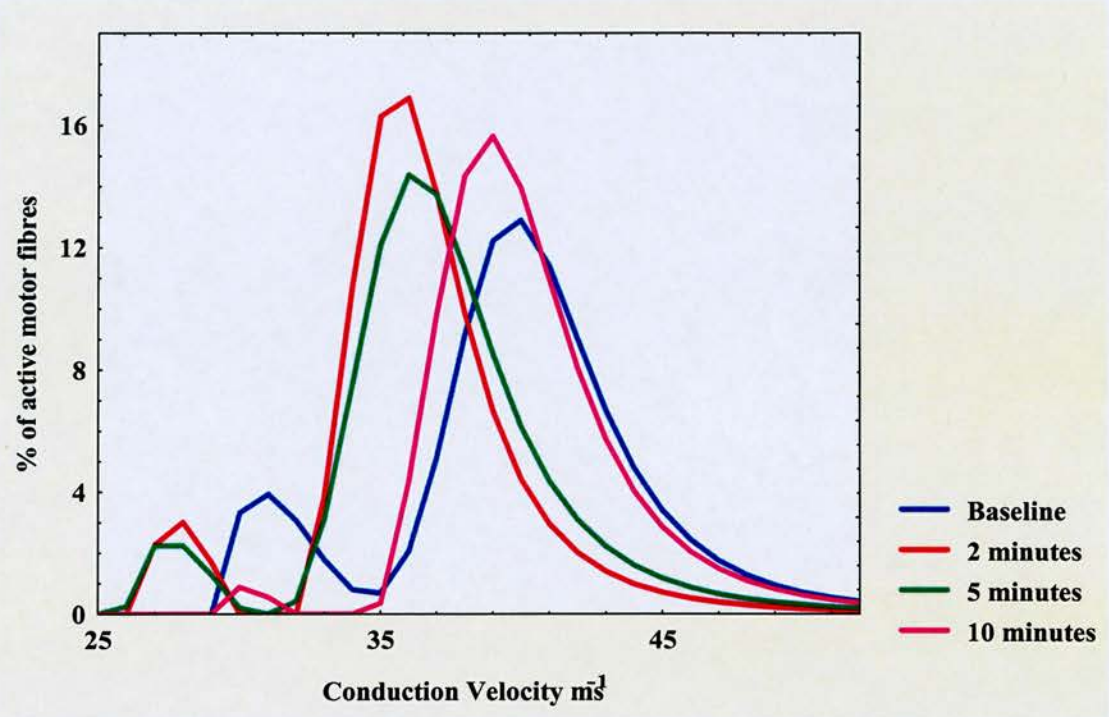


Figure 19

motor CV_{Dist} during recovery from ischaemia 0–10 minutes

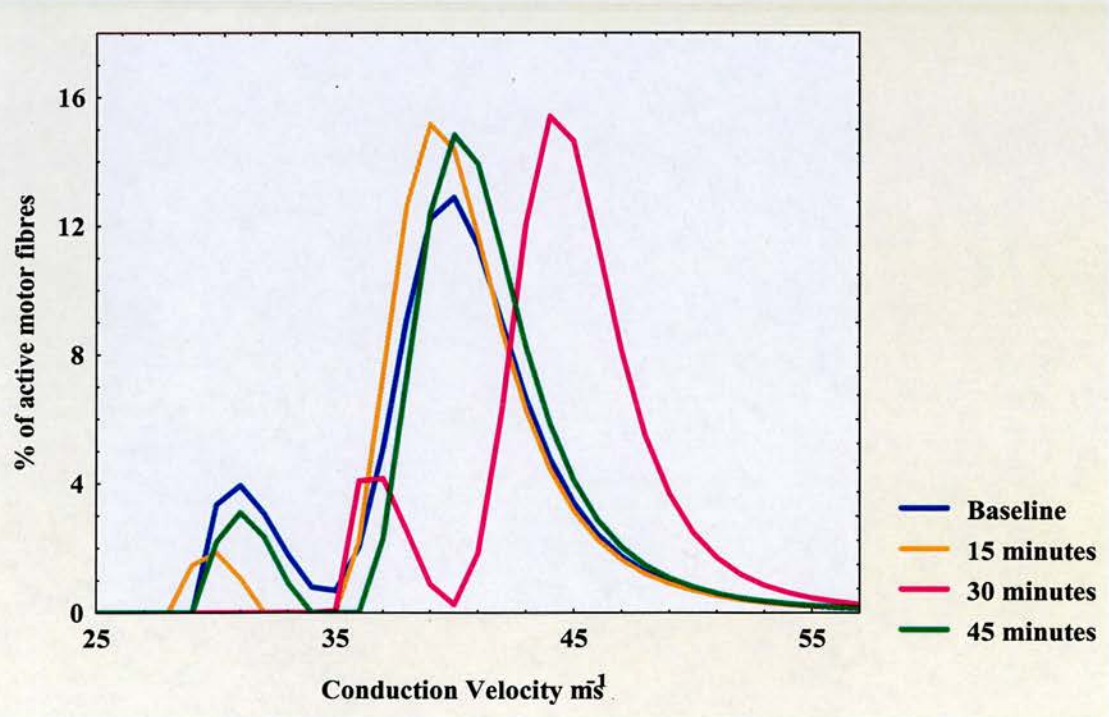


Figure 20

Motor CV_{Dist} during recovery from ischaemia 15–45 minutes

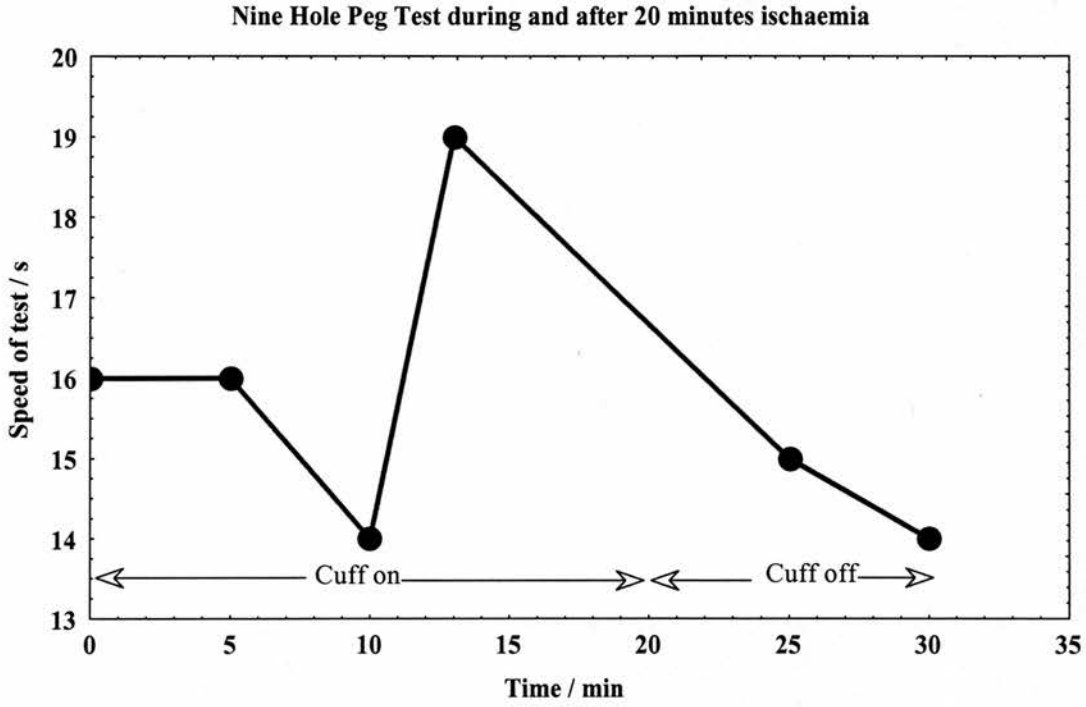


Figure 21

Nine hole peg test during and after ischaemia for 20 minutes. The y-axis represents the number of seconds taken to complete the test correctly at each time on the x-axis.

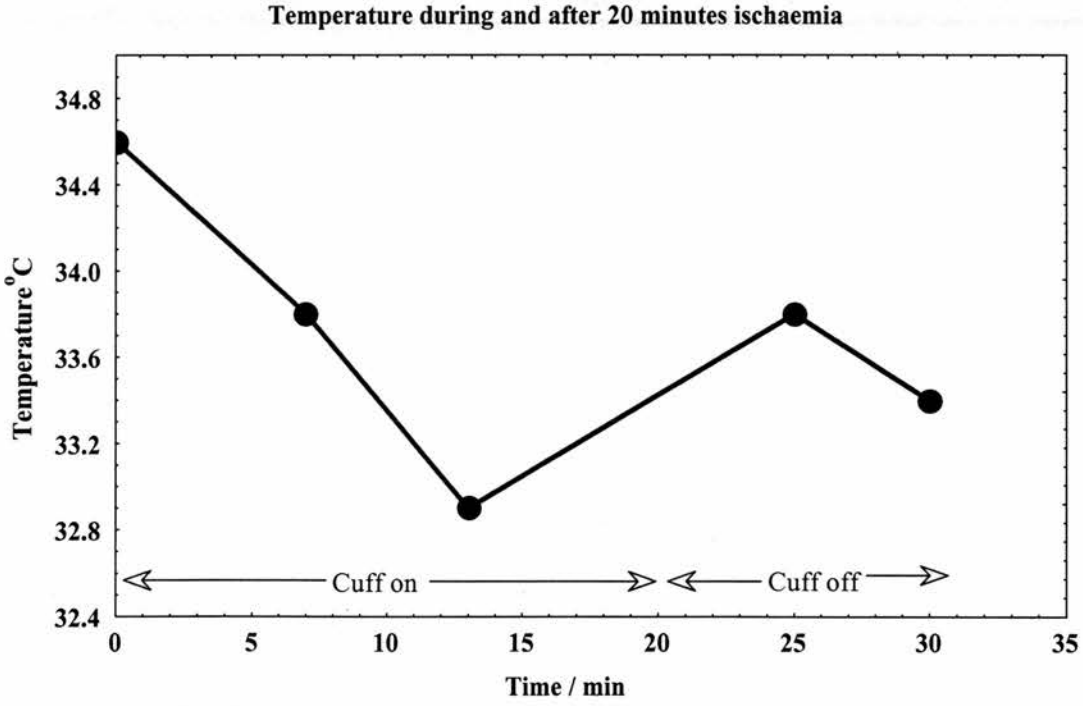


Figure 22

Temperature during and after ischaemia for 20 minutes

A similar pattern was evident with the sensory CV_{Dist}. Progressive shifting of the sensory CV_{Dist} profile occurred with advancing ischaemia, up to a point where it was no longer possible to record sensory action potentials. This happened after around 20 minutes of occlusion and coincided with complete anaesthesia. After deflation of the sphygmomanometer cuff a throbbing sensation was reported followed by intense painful paraesthesiae. This period coincided with the subnormal period evident in the recording taken at 3 minutes. An overshoot was also seen during the recovery of sensory CV_{Dist} recordings. Also of note, was the residual leftward shift seen in the sensory CV_{Dist} at 60 minutes. Despite subjective 'normal sensation' occurring within minutes of restoration of circulation, latent subclinical changes continued to be evident long after this. The drop in temperature that occurred was less severe than that seen during motor recording and again no adjustment for temperature was made in this range.

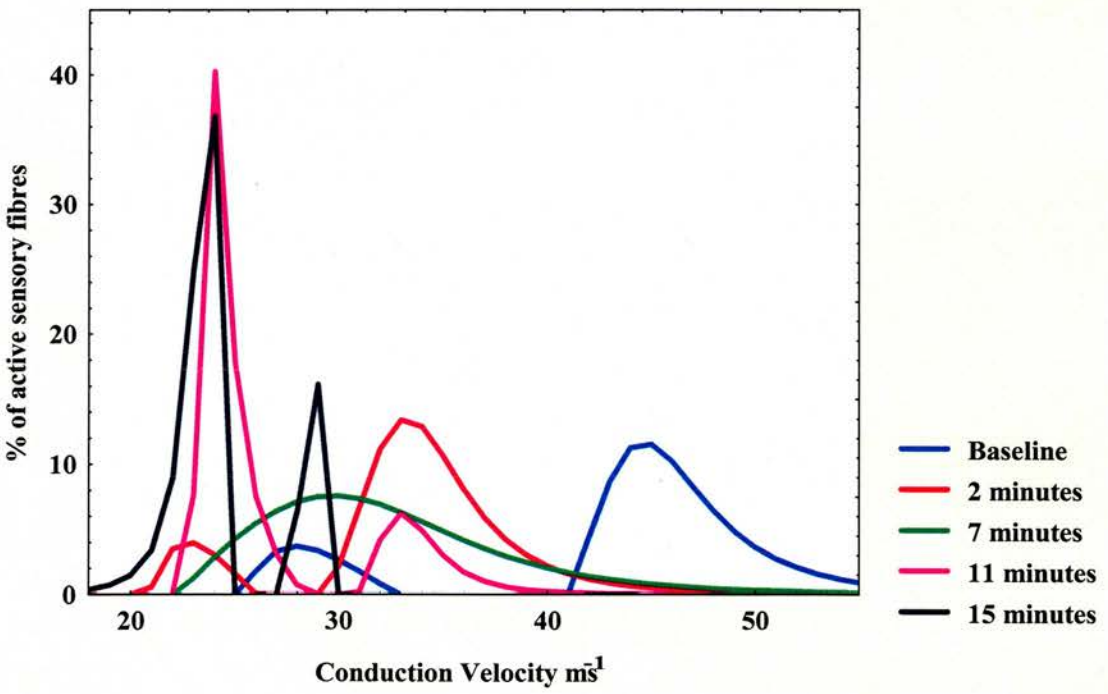


Figure 23

Sensory CV_{Dist} during induction of ischaemia 0–15 minutes

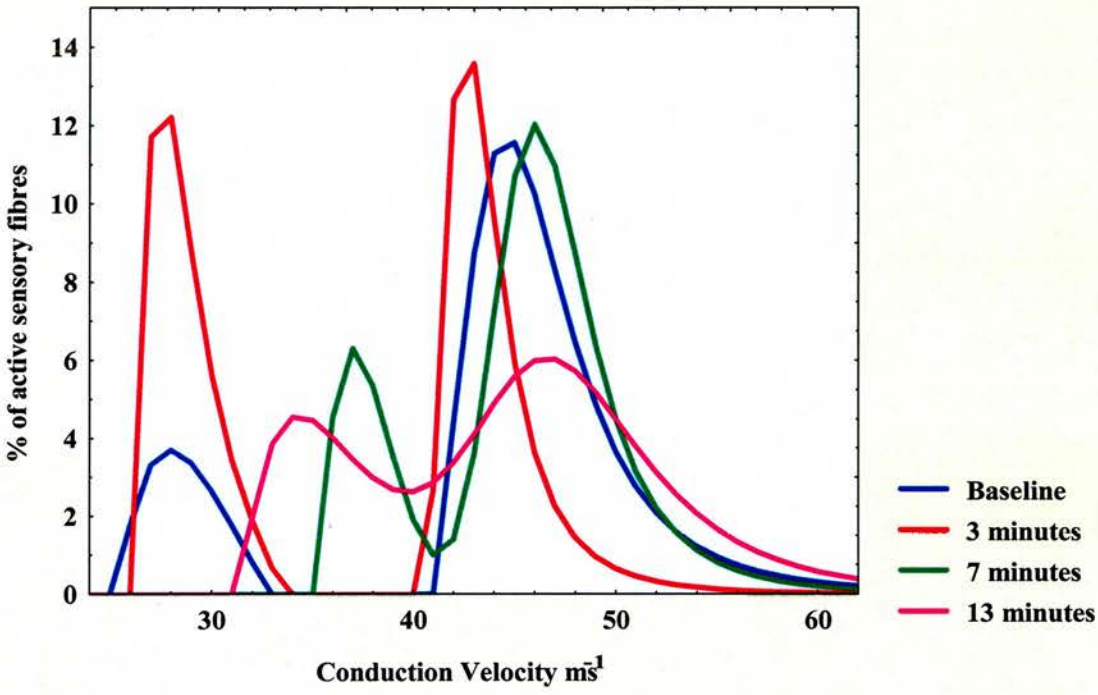


Figure 24

Sensory CV_{Dist} during recovery from ischaemia 0–13 minutes

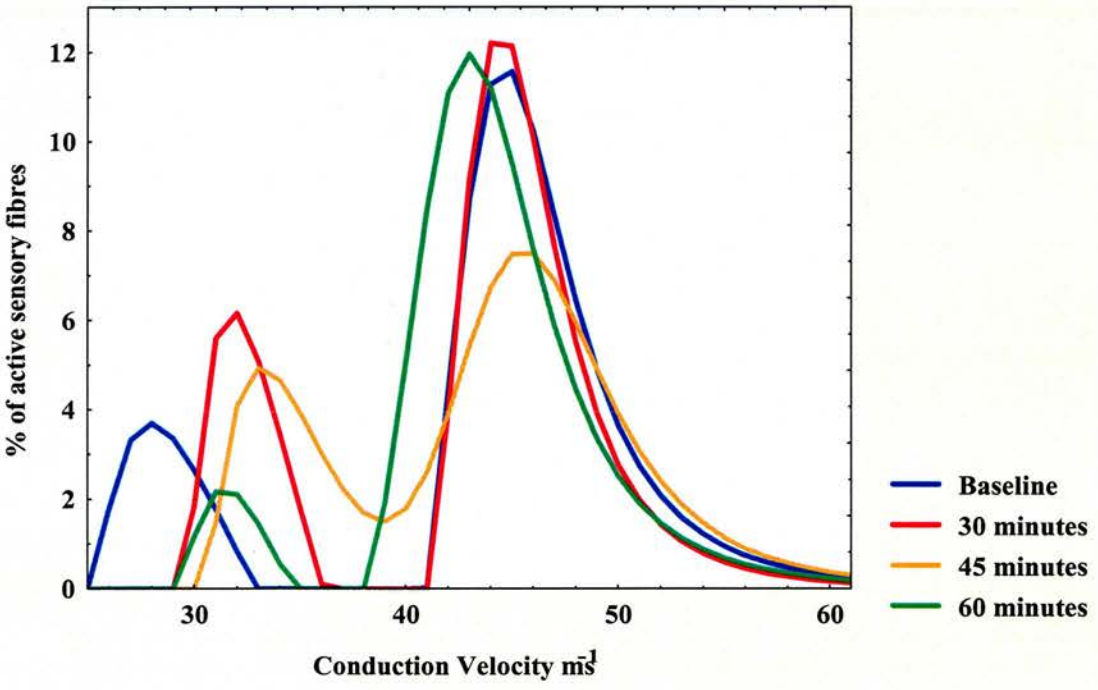


Figure 25

Sensory CV_{Dist} during recovery from ischaemia 30–60 minutes

In conclusion, these basic experiments offer considerable encouragement about the possibilities for using the CV_{Dist} test in clinical diagnosis and follow-up. The apparatus, beyond a basic machine for nerve conduction studies, is cheap and disposable. The software obtainable with most conventional machines nowadays offers enough 'customizable' programming to allow some version of the CV_{Dist} test to be possible assuming that the machine supports dual and independent stimulators. However more sophisticated software produced commercially would allow the test to be streamlined further. Nevertheless it was found that adequate testing could be carried out within a twenty minute period and this seems worthwhile. As concluded in Chapters 3 and 4, CV_{Dist} would almost certainly have more to offer in the diagnosis and follow-up of neuropathies or neurapraxia and axonotmesis rather than in transecting nerve injuries. However it would undoubtedly be of use in the follow-up of repaired nerve injuries as it may be expected to reflect changes taking place with time with a greater sensitivity and accuracy than is hitherto available among conventional objective tests.

CHAPTER 7 — THE BRACHIAL PLEXUS

OBSTETRICAL BRACHIAL PLEXUS PALSY

OBSTETRICAL brachial plexus palsy (OBPP) is a rare but debilitating injury occurring at the time of delivery as a consequence of traction on the brachial plexus. OBPP was first described by Smellie as long ago as 1765 but was not confirmed as having been brought about by the mechanics of the process of delivery until it was again described by Duchenne in 1872. It was not until the last quarter of the nineteenth century that the condition was formally characterized by the work of Erb and Klumpke, (Birch, Bonney, & Wynn-Parry 1998).

The most famous case of OBPP is undoubtedly that of Kaiser Wilhelm II of Germany who was born in 1859. The historical significance of his condition has recently been considered in fascinating detail by Röhl, (Röhl 1998): the lesion was probably what today would be classified as either Tassin group III or group IV (Gilbert & Tassin 1987) and was treated on the advice of Bernhard von Langenbeck, Professor of Surgery in Berlin (a 'long-winded, garrulous and unpleasant pompous ass' according to Crown Princess Victoria), by the insertion of the child's arm, twice-weekly for half an hour, into the abdominal cavity of a freshly killed hare. This treatment, which was supposed to 'transfer the warmth and vigour of the [unfortunate] animal to the undernourished arm', turned out to be a disappointment (Röhl 1998). Nevertheless as is so often the case it focused attention upon this rare but serious condition.

The incidence of OBPP in the United Kingdom is unknown but is thought to be between 0.1% and 4% of live births and to be rising (Birch, Bonney, & Wynn-Parry 1998). Of the large number of studies, those of Gilbert (Gilbert 1995a; Gilbert 1995b) and of Slooff and Blauuw (Slooff 1995; Slooff & Blauuw 1995) are the most useful in

defining both incidence and risk factors. Small mothers, large babies, the somewhat ill-defined condition of shoulder dystocia and breech delivery are implicated, but their interaction in giving rise to the injury is complex (Birch, Bonney, & Wynn-Parry 1998). There is, however no doubt that the incidence is rising and this rise has been shown to be due to the increasing birth-weight of babies, presumably as a result of improving standards of living (Power 1994). More controversial has been the implication of ill-informed maternal choice tending towards a preference for home delivery, (Birch et al 1998; Slooff 1995; Slooff & Blauuw 1995).

The strategy for management of OBPP has been made difficult by the problem of establishing the precise nature of the injury. The work of Erb and Klumpke in the late nineteenth century divided OBPP into two characteristic patterns that bear their names. In practice there is a range of injury and severity so that application of the traditional strategies for managing nerve injuries is not always appropriate. The least severe injuries where the nerves, including the endoneurial sheath are in continuity (*neurapraxia* or *axonotmesis* in the Seddon classification , I^{st} and II^{nd} degree injuries in the Sunderland classification), require expectant treatment and a good outcome can be seen in most cases. In the more severe cases where there has been rupture of nerves (*neurotmesis,* IV^{th} or V^{th} degree injury), the need is for early operative repair by nerve-grafting or subsequent musculo-tendinous reconstructive procedures. There is a spectrum of injury between these two points and it can be difficult to predict from clinical, electrophysiological and imaging studies the exact nature of the injury. Assessment is difficult because different parts of the plexus can be affected by different grades of injury.

There remains the problem of the traction injury where all of the neural elements are ruptured but the epineurium is preserved. This is the Sunderland IV^{th} degree lesion

(Sunderland 1951) often referred to as the 'lesion in continuity' though it should be appreciated that this is a rather non-specific term which could refer equally to lesions of Sunderland type I, II, III or IV.

Traction to the brachial plexus may result in avulsion of nerve roots from the spinal cord (pre-ganglionic injury) or damage more distally to the trunks, divisions, and cords (post-ganglionic injury). The severity of post-ganglionic injury can be graded using Seddon's (Seddon 1943) or Sunderland's (Sunderland 1978; Sunderland 1990; Sunderland 1951) classifications. As it is impossible clinically and neurophysiologically to differentiate between types I, II, III and IV injuries, Mackinnon and Dellon (MacKinnon & Dellon 1988a; MacKinnon & Dellon 1988b) have advocated waiting for a period of three months after injury. This is because it might be expected that, by that time, injuries of types I, II and III would have started to have shown an orderly progression of recovery and therefore could be identified clinically or neurophysiologically.

There is no doubt that the best functional outcome occurs when spontaneous recovery takes place but waiting is not without its pitfalls. Muscles atrophy and undergo fibrosis and joints suffer contractures unless frequent exercises are performed. Waiting may also be detrimental if the regenerative potential declines with age. It is possible to postulate that reorganization of the nervous system occurs more fully in the new-born than in the older animal owing to the greater plasticity of nerve connections at a younger age.

How long it is appropriate to wait in those cases where no significant improvement appears to be taking place before making the decision to operate is a difficult question. The detrimental effects of delay in repair have to be weighed against the possibility of spontaneous recovery and the risks of operation at a very

young age. The most widely accepted strategy is to carry out operation if elbow flexion has not recovered after 3 to 6 months except in Sunderland IVth degree cases when operation is carried out as early as it can be safely performed (Birch, Bonney, & Wynn-Parry 1998; Bonnard & Narakas 1995; Glasby et al. 1986a; Glasby et al. 1986b; Glasby, Fullarton, & Lawson 1997; Hakamada et al. 1982; Hems, Clutton, & Glasby 1994b; Hems & Glasby 1992b).

This strategy (Smith 1996; Smith 1998) is widely used in OBPP but a variety of both laboratory and clinical investigations in other fields of nerve repair has established the seemingly contradictory general principle that the earliest repair, especially by means of nerve grafts, is associated with the best level recovery (Fullarton, Glasby, & Lawson 1998; Glasby, Fullarton, & Lawson 1997; Glasby, Fullarton, & Lawson 1998; Lawson & Glasby 1995a; Lawson & Glasby 1995b). Moreover, Narakas has emphasized the need for exploration and repair *as early as possible* in *adult* brachial plexus injuries (Bonnard & Slooff 1999; Narakas 1987; Narakas 1991; Narakas 1993).

In operating on OBPP in neonates one is beset by a number of problems which are not encountered in adults or older children. Some of these, such as thermoregulation, are of a general nature and others are specific to the operation for brachial plexus repair. In undertaking such a procedure in a neonate access is more restricted: this may lead to longer anaesthetic times and any inevitable blood-loss represents a greater proportion of the total circulating volume. Scarring at the site of injury has been found to be worse in neonates (Birch, Bonney, & Wynn-Parry 1998; Birch 1992a; Birch 1992b) and this too results in prolonged dissection and further bleeding. The harvesting of grafts is a further problem. In the upper limb the medial cutaneous nerve of the forearm, superficial radial nerve and the lateral cutaneous nerve of the forearm

have all been described as donor nerve for grafts though it has been suggested that these together provide only enough nerve to repair one or two ruptures (Birch et al. 1998). Any additional donor nerve must be supplied by the sural nerve(s) and there are further problems of access, disfigurement and blood loss involved in obtaining these nerves. Mackinnon and Dellon (MacKinnon & Dellon 1988b), following Narakas, have stated that the most important aims of brachial plexus repair should be to reestablish ***shoulder abduction***, ***elbow flexion*** and the restoration of ***sensation in the medial border of the forearm and hand***. The last of these is clearly not possible if the appropriate cutaneous nerves have been sacrificed and thus an alternative strategy relies more heavily on provision of grafts from the sural nerves.

There is thus a strong theoretical case for the use of non-neural graft material though it is acknowledged at present that the results of using such grafts are generally poorer than repair with nerve autografts. The freeze-thawed muscle graft (FTMG) has been used in some instances as a means of repairing a nerve defect *en masse* but has not gained the popularity that was hoped for (Norris et al. 1988; Pereira et al. 1991). Early reports from laboratory studies (Davies et al. 1987; Fullarton et al. 2000; Gattuso et al. 1988; Gattuso, Glasby, & Gschmeissner 1988; Glasby et al. 1986a; Glasby et al. 1986d; Glasby, Gschmeissner, Hitchcock, & Huang 1986a; Glasby, Gschmeissner, Hitchcock, & Huang 1986b; Glasby 1990; Glasby, Gattuso, & Huang 1988; Myles, Gilmour, & Glasby 1992; Myles & Glasby 1992; Stirrat, Birch, & Glasby 1991; Thomas et al. 1994), were encouraging but emphasized an absolute need to prepare the nerve with adequate freezing to –196°C followed by complete thawing in an hypotonic medium (water). A number of workers have attempted to use muscle autografts where no freezing and thawing or less than adequate preparation of the graft was employed (Calder & Norris 1991). It is hardly surprising that the results

obtained by these workers were poor. As a consequence of poor scientific method and over-generous editorial practices the consequence has been an understandable reluctance on the part of surgeons to attempt this technique even in situations where its use may be likely to be acceptable. Instinctively, most surgeons would opt for either interfascicular or cable grafts as the preferred technique for bridging either a long or a short gap. When multiple roots are involved it might be thought worthwhile to repair the less important ruptures with short (<5 cm) FTMGs if one has run out of nerve autograft or if the projected result does not appear to justify further intervention to obtain nerve (Bonnard & Slooff 1999).

A considerable quantity of both experimental and clinical work has been published about OBPP. The overwhelming impression which is gained from an examination of this work is its heterogeneity and a consequence of this is that there is no clear paradigm of how to manage these children. The heterogeneity of the injury itself must be blamed for much of this as it is often very difficult to assemble an adequate number of similar cases to make any form of quantitative evaluation possible (Narakas 1993). The natural consequence of this is a parallel diversity of diagnostic methods (Alnot & Blaauw 1995; Birch, Bonney, & Wynn-Parry 1998; Birch 1992b; Birch 1993; Francel et al. 1995; van Daalen et al. 1993), treatments (Alnot & Blaauw 1995; Birch, Bonney, & Wynn-Parry 1998; Geutjens, Gilbert, & Helsen 1996; Gilbert 1995a; Gilbert 1995b; Gilbert & Tassin 1984; Gilbert & Tassin 1987; Laurent et al. 1993; Narakas 1991; Tonkin, Eckersley, & Gschwind 1996) and measurements of outcome (Alnot & Blaauw 1995; Bellew et al. 2000; Gilbert 1995b; Slooff & Blauuw 1995) so that clear guidelines never emerge. This is a problem common to all studies of peripheral nerve injury and its management. The other major reason for confusion lies in the degree to which authors have extrapolated from adult BPP management to

OBPP. In particular there is a welter of confusion over the timing of repair. Almost all authors agree that it should be 'early' but few of them agree on what the term 'early' means. For example, Tonkin et al (Tonkin, Eckersley, & Gschwind 1996) advise exploration and repair within two weeks of brachial plexus injury while Geutjens et al (Geutjens, Gilbert, & Helsen 1996) showed a 'favourable' outcome after surgery at three months and Michelow et al (Michelow et al. 1994) made the point that if elbow flexion had returned by three months, this correlated well with recovery by one year. Laurent et al (Laurent, et al 1993) recommended surgical reconstruction if there was no improvement by four months while Mehta et al advocate operation after six months (Mehta, Banerji, & Tripathi 1993). Birch and Bonney (Birch, Bonney, & Wynn-Parry 1998) take a less rigid view of timing and argue that the nature of the injury as assessed by the Gilbert and Tassin classification (Gilbert & Tassin 1987) is important. Within the time period of two to four weeks after injury simple conduction block will have recovered and the Tassin classification is meaningful at this time. The Tassin classification does not, however, concern itself with *e.g.* the pure Klumpke lesion and thus must not be considered to be an absolute indicator for operation.

Gilbert and Tassin's classification of OBPP is based on clinical features, which is simple, easily applied and offers a broad guide to prognosis. It is as follows:

- Group 1 The 5th and 6th cervical nerves are damaged. There is paralysis of deltoid and biceps. About 90% of children will go on to have full spontaneous recovery with clinical evidence of recovery being obvious at 2 months.
- Group 2 The 5th, 6th and 7th nerves are damaged. The long flexors of the hand are working from the time of birth but there is paralysis of extension

- of elbow wrist and digits. Only 65% of these children will go on to have full spontaneous recovery. Recovery is slower with activity in deltoid and biceps only becoming apparent between 3 and 6 months.
- Group 3 Paralysis is virtually complete. There may be some flexion at the fingers at, or shortly after birth. Full spontaneous recovery occurs in less than 50% of these children. Most are left with substantial impairment of shoulder and elbow function.
- Group 4 The whole plexus is involved. Paralysis is complete. The limb is atonic and there is a Claude Bernard – Hörner syndrome.

The Gilbert and Tassin classification should be applied at between 2 and 4 weeks after birth when cases of conduction block should have recovered.

At operation the damage to the plexus is defined. Where post-ganglionic rupture of nerves has occurred, nerve grafting is performed. In cases where nerve roots have been avulsed, the current strategy is to reconstruct the plexus with grafts from the roots which have not been avulsed and to perform nerve transfers from the accessory and intercostal nerves. Fortunately, it is very unusual for all the roots to be avulsed.

There is a prevailing view among clinicians that root avulsions cannot be repaired but there is experimental evidence that ventral roots can regenerate (Carlstedt & Noren 1995; Carlstedt 1995; Culheim, Carlstedt, & Risling 1999; Fullarton, Lenihan, Myles, & Glasby 2000; Fullarton et al. 2001; Fullarton et al. 2002; Gilbert & Tassin 1984; Gilbert & Tassin 1987; Glasby & Hems 1995; Hems, Clutton, & Glasby 1994; Hems & Glasby 1992). In our laboratory is has been shown that avulsed ventral roots can regenerate through a muscle graft and conduct action potentials . Reinnervation of muscles has been demonstrated (Carlstedt et al. 1986; Hems, Clutton, & Glasby

1994). Carlstedt (Carlstedt 1991; Carlstedt et al. 1993) has reported recovery after reimplantation of motor roots in cases of traction injury to the adult brachial plexus. The dorsal roots central to the dorsal root ganglion have an uncertain regenerative potential, and it is doubtful whether they can make functional connections in the dorsal horn.

The objective of the experiments described here has been to use the ovine model to answer first the question of how and at what time to treat a clearly *identified* Sunderland type IV traction injury to establish whether there was a difference in the degree of recovery when new-born animals were compared with adults. Additionally the timing of the operation has been considered. The model for this part of the experiment was the C6 root of the brachial plexus in the sheep and new-born lamb which previous work has shown to be a good model for human nerve repair (Glasby, Hems, & Pell 1992; Hems, Clutton, & Glasby 1994; Hems & Glasby 1992).

The second part of the study has been concerned with a model of the avulsion injury in new-born lambs and adult sheep.

A single ventral root (C6) was avulsed to provide the clearest and most simple example of the injury and to reduce disability in the animal.

The C6 brachial plexus root in the sheep is equivalent to C5 in the human: it uniquely innervates supraspinatus and contributes to the innervation of infraspinatus and the formation of the musculocutaneous and median nerves. Unlike the situation in the human, the loss of this root in the sheep is not a great impediment to its normal activity. Thus, since it is also a root most commonly injured in OBPP it is, in theory, an excellent experimental model.

In assessing outcome some of the standardized techniques for measuring the indices of nerve regeneration described earlier in this thesis were used. The results have been

compared with those of a similar study in which repair of complicated neurotmesis of the median nerve more distally in the arm was considered at similar times (Fullarton, Glasby, & Lawson 1998; Glasby, Fullarton, & Lawson 1997; Glasby, Fullarton, & Lawson 1998; Lawson & Glasby 1995b; Lawson & Glasby 1998; Sherrington 1892; Sherrington 1894).

Experimental Methods

Traction injury model

Cohorts of year-old female Scottish Black-face sheep each weighing approximately 40 kg or of new-born Black-face lambs were used. They were divided into groups as follows:

Cohort 1 Age of Animal

Groups 6 animals per group:

1. Controls — no treatment
2. Sheep — C6 traction injury and repair with cable graft
3. Lambs — C6 traction injury and repair with cable graft

Cohort 2 Method of Repair

Groups, 6 animals per group:

4. Controls — no treatment
5. Sheep — C6 traction injury and repair with cable graft
6. Sheep — C6 traction injury and repair with FTMG
7. Lambs — C6 traction injury and repair with cable graft
8. Lambs — C6 traction injury and repair with FTMG

Cohort 3 Timing of Repair

Groups 6 animals per group:

9. Lambs — C6 traction injury and repair with cable graft — immediate repair
10. Lambs — C6 traction injury and repair with FTMG — immediate repair
11. Lambs — C6 traction injury and repair with cable graft — repair at 30 days
12. Lambs — C6 traction injury and repair with FTMG — repair at 30 days

The adult animals were chosen to correspond as nearly as possible to humans of about 15 – 20 years of age. There are no clear data to compare human and sheep development so this choice had to be somewhat arbitrary and should not be considered too precise. These names were chosen to reflect the age of the animal *at the time of creation of the brachial plexus injury and of the operation for its repair.* At the time of assessment, one year after the operation, all of the animals were adult but for the purpose of the present account the terms *lamb* and *sheep* are still used. Although in human practice, obstetric brachial plexus injuries usually occur at the moment of birth, such would have been logistically impossible in the present experiments and so lambs of up to one week of age were used. It is unlikely that maturation of the peripheral nervous system during this brief, early period would have taken place in such a way as to invalidate the model (Hakamada, Kumagai, Watanabe, Koike, Hara, & Miyazaki 1982).

General anaesthesia in each animal was induced intravenously using a combination of midazolam (Hypnovel; Roche) and etomidate (Hypnomidate; Janssen) The animals were intubated with a cuffed endotracheal tube and their lungs were ventilated with a fresh gas flow of oxygen and of nitrous oxide. Drug dosages and gas flow rates were determined according to the weight of the animals. Anaesthesia was mainained with 1–2% halothane. Neuromuscular blockade was not used. During the operation, body

temperature was maintained with a heating blanket and maintenance fluids were given intravenously. Respiratory rate, SaO_2, electrogardiogram, arterial blood pressure, central venous pressure and rectal temperature were monitored throughout the operation.

The surgical approach to the left brachial plexus was the same for both lambs and sheep. The animal was placed on the operating table on its right side with its neck extended. The entire neck and 'clavicular' area (the sheep has no clavicle) were shaved and prepared first with depilatory cream and then with a Betadine solution in alcohol. An incision was begun in the mid-line over the spinous process of C6 and extended through the posterior triangle of the neck down to a point over the anterior (superior in the human) border of the scapula. It was important not to incise or elevate skin over supraspinatus as this would later be a site for transcutaneous electrophysiological recording. Elsewhere, subcutaneous connective tissue was separated and platysma and splenius cervicis were divided at their attachments to the scapula. The anterior border of the scapula was elevated and deep dissection revealed either the suprascapular nerve or the upper trunk of the brachial plexus both of which could be readily and positively identified. By tracing these nerves centrally, the C6 root of the plexus could be identified and isolated. In the sheep the C6 root is equivalent to C5 in the human (Hems, Clutton, & Glasby 1994; Hems & Glasby 1992): it uniquely innervates supraspinatus and contributes to the innervation of infraspinatus and the formation of the musculocutaneous and median nerves. Its loss it not a great impediment to the normal activity of the sheep. Thus, since it is also equivalent to a root most commonly injured in OBPP it is an excellent experimental model.

In order to produce a model for the Sunderland type IV lesion it was necessary to obliterate all neural and connective tissue other than the epineurial sheath. In traction injury of the brachial plexus, this comes about by means a sudden distracting force applied along the axis of the nerve or indirectly by increasing the angle between the neck and the shoulder which is often impacted at delivery. It is virtually impossible, in the laboratory, to achieve a consistent lesion using violent methods of this sort: for the present experimental model a means was sought which would produce a consistent injury of identical morphology and extent at the expense of a precise re-enactment of the traumatic insult. A crush injury was produced first by clamping the nerve by means of two adjacent curved Dunhill clamps which had been applied with their convex faces back-to-back and closed to the third point of the ratchet. A distracting force was then applied between the two clamps by pressing and rotating the convex surfaces against each other until an extension of the nerve of 0.5 cm was seen between them. This force was maintained for a 30 second period. The procedure resulted in a crushed segment of nerve approximately 1.5 cm long. This technique had been found in preliminary studies to produce complete destruction of all neural tissue and perineurium within the crushed segment with preservation of the epineurial sheath. In all cases subsequent histological examination of the excised site of injury showed a morphological picture which was consistent with a Sunderland type IV lesion.

Once the lesion had been made, the operating microscope was introduced and the lesion resected. The nerve stumps were trimmed back to what appeared to be normal nerve when viewed with the aid of the operating microscope. In every case this led to a gap of between 1.5 cm and 2 cm in length. The nerve was repaired by means of a three-strand cable graft obtained from branches of the cutaneous nerves which were to

be found lying superficially in the neck. In most cases it was possible to identify one large fascicle in the C6 root and this was repaired using one of the strands of cutaneous nerve. The remainder of the C6 root was repaired using two further strands of cutaneous nerve sutured with interrupted 10/0 or 11/0 polyamide interrupted sutures to the epineurium of the plexus root. In all cases a minimum number of sutures was used and the suture line was reinforced with a small quantity of fibrin glue (Tisseel, Immuno, U.K.) . Two 'Ligaclips' (Ethicon U.K.) were placed as markers of the site of repair and the wound was closed in layers using absorbable suture material (Vicryl, Ethicon U.K.). The animals recovered rapidly from the operation; the lambs were returned immediately to their mothers and all animals were eventually returned to their farm where they remained for one year. They were monitored periodically to assess recovery and general health.

At the time of assessment anaesthesia was induced and maintained as previously. No neuromuscular blockade was permissible as this would have interfered with the electrophysiological measurements.

With the sheep lying on its right side, the left foreleg was extended and secured. The skin was shaved over the left upper limb in the form of a triangle the medial border of which extended from the C2 spinous process to the T4 spinous process in the mid-line. The caudal border extended from the T4 spinous process laterally to the inferior border of the scapula and the third border extended from the inferior border of the scapula to the C2 spinous process. The shaved skin was washed with warm water and any remaining hair was removed with a hand-held razor. The skin was then cleaned with Betadine in alcohol to remove excess oil and dirt.

Assessment

A detailed discussion of these tests and the rationalization for their use is to be found in Chapter 3.

All electrophysiological tests were in, general, carried out as described earlier.

Jitter (TSJ) measurement in the supraspinatus muscle (SSM) was the first electrophysiological variable to be assessed. As in the human, this muscle is uniquely innervated by the suprascapular nerve which mainly contains fibres derived from the uppermost root of the brachial plexus (C5 in the human and C6 in the sheep). The muscle is easily recognized through the skin and is small but bulky allowing needle placement to be performed with ease and without any risk of confusion with adjacent muscles. Two unipolar needle electrodes (Medelec MF 37, Old Woking, Surrey UK) were so used to stimulate the terminal motor axons of the suprascapular nerve in SSM. The cathode and inserted into the SSM at the motor point which was found to be located approximately 3 cm distal to the superior border of the scapula in the centre of the muscle belly. The anode was placed 2 cm superior to the cathode in the centre of the SSM muscle belly. A single-fibre electrode (SF-EMG - Medelec, Old Woking, Surrey UK) was inserted into the twitching muscle approximately 1.5 to 2.5 cm distal to the cathode and moved around until a stable motor unit action potential was recorded.

In calculating the jitter value of the SSM, the mean consecutive difference (MCD) and the mean sorted difference (MSD) were used after recording from 20 different SSM sites for each animal. The MCD was taken as the jitter value for each site provided the MCD/MSD ratio was 1.25 or below; otherwise the MSD was taken as the jitter value (Ekstedt & Stålberg 1973; Kimura 2001; Stålberg 1990; Stålberg & Trontelj

1994; Stålberg, Trontelj, & Mihelin 1992) the average of all jitter measurements was the *mean jitter* for that animal.

The C6 root of the plexus was identified and isolated as above. The nerve, though relatively short was nevertheless long enough to accommodate the two stimulating electrodes necessary for segmental velocity recording. However when dealing with short segments it is always advisable to ensure clear 'take-offs' and care in finding the motor-point is repaid. As the animal has thick skin in the shoulder region, it was thought appropriate to record CMAPs directly from the exposed muscle belly. An incision was made made directly over the motor point of SSM and a 6 mm Ag/AgCl disc recording cathode was positioned directly on the SSM belly. A second incision was made at the inferior border of the scapula directly over its spine and a similar anode was placed over the spine of the scapula.

The maximum conduction velocity (CV_{max}) was obtained by dividing the distance between the stimulating electrodes by the difference in the latencies of the take-off points of the compound muscle action potentials (CMAP) in SSM obtained after stimulation at each site. Minimum conduction velocity (CV_{min}) and refractory period (RP) were measured next using a variation on the 'collision technique' which has been described elsewhere (see chapter 3 also: (Gilchrist et al. 1998; Hopf, Lowitzsch, & Galland 1976; Lenihan et al. 1998; McDowall K.L. et al. 1998).

When physiological measurements were complete, samples of nerve tissue distal to the graft were taken and process used for morphometric study as described in Chapter 3, above.

Statistical analysis is likewise discussed in Chapter 3. As in the present study there was the possibility of considering 3 independent variables (factors) the Factorial ANOVA (Statistica, Statsoft Ltd Bedford UK) algorithm was applied to the results in

order to assess if and where overall effects could be identified. This is of particular interest in considering how the results may be interpreted in the context of human nerve injuries. It was not thought necessary to consider adult sheep with 'late' repair because previous work had shown them to do particularly badly and in any case such a study was not appropriate as a model for OBPP.

Avulsion Injury Model

Six one year-old female Scottish Black-face sheep each weighing approximately 40 kg were used and designated the *sheep group*. A further six one week-old lambs were designated the *lamb group.* These names were chosen to reflect the age of the animal *at the time of creation of the root-injury and of the operation for its repair.* A further six normal one year old sheep were used as the *control or intact group.*

Groups: 6 animals per group:

13. Controls — no treatment
14. Sheep — C6 ventral root avulsed and repaired with FTMG
15. Lambs — C6 ventral root avulsed and repaired with FTMG

General anaesthesia in each animal was induced intravenously using a combination of midazolam (Hypnovel; Roche) and etomidate (Hypnomidate; Janssen) at dosages determined by body weight. Maintenance fluids were given intravenously. The animals were intubated with a cuffed endotracheal tube and their lungs were ventilated with a fresh gas flow of oxygen, nitrous oxide and 1-2% halothane. Neuromuscular blockade was induced. Body temperature was maintained with a heating blanket. Respiratory rate, SaO_2, electrogardiogram, arterial blood pressure, central venous pressure and rectal temperature were monitored throughout the

operation. A single dose of cefuroxime (Zinacef, Glaxo Laboratories Ltd, Greenford, UK) 750mg intravenously was given for antibiotic prophylaxis at the time of induction of anaesthesia.

All surgery was carried out in an operating theatre under sterile conditions. The animals were placed prone and in the 1 year old sheep the head was fixed securely in a Mayfield neurosurgical head-holder. For the lambs, the paediatric horseshoe attachment for the Mayfield head-holder was used and the head was secured to it with Elastoplast tape. The back of the neck was shaved and prepared first with depilatory cream and then with Betadine solution. The laminae of C5 and C6 were exposed through a midline skin incision using standard neurosurgical technique. A left hemilaminectomy was performed at these levels to expose the dura and the C6 root sleeve.

The dura was opened and the rootlets making up the C6 root were identified. The lateral edge of the dura was retracted in order to expose the ventral rootlets which were then avulsed from the cord using a Rhoton nerve hook. The dorsal rootlets were also avulsed but the dorsal root ganglion remained intact.

A piece of muscle was taken from erector spinae and an autologous freeze-thawed muscle graft was prepared according to the technique previously described (Glasby 1990) The graft was trimmed into a wedge shape, with the fibres longitudinally aligned, so as to fit into the space between the anterolateral surface of the spinal cord and the posterior surface of the vertebral body. The ends of the avulsed ventral rootlets were trimmed and abutted to the lateral surface of the muscle wedge. They were secured with fibrin glue ('Tiseel'; Immuno; Vienna; Austria). The dura was repaired with 10/0 polypropylene sutures and the wound was closed in layers using absorbable suture material (Vicryl, Ethicon U.K.). A second dose of cefuroxime was

given prior to recovery. The animals recovered rapidly from the operation; the lambs were returned immediately to their mothers and all animals were eventually returned to their farm where they remained for one year.

After one year, electrophysiological and histological examinations were carried out. The animals were anaesthetized as described above but without the use of neuromuscular blockade. The old incision was opened and a complete laminectomy performed at the previously operated level. The incision was then extended laterally to expose the C6 root from the spinal cord to the brachial plexus. A craniectomy was performed to allow direct stimulation of the motor cortex. The *sheep* and *intact* groups were examined in exactly the same way.

Electrophysiology:

All electrophysiological tests were carried as before. Because of the very proximal nature of the injury in this model some modifications were necessary. For measurement of CV_{max} a bipolar needle electrode, insulated except for the last 1mm, was placed into the anterior horn of the spinal cord at the C6 level. Square wave stimuli (0.1msec, 10V) were applied. The shoulder girdle muscles were observed for contraction. A 0.3mm palladium wire bipolar recording electrode was placed on the C6 root as far laterally as possible and compound motor action potentials (CMAP) were obtained. Sixteen action potentials were averaged in each case. The distance between the recording and stimulating electrodes was measured and $CV_{\max}$, $CV_{\min}$ and CV_{range} calculated.

Central motor latency (CML) was measured by placing a stimulating anode on the contralateral motor cortex, exposed through a craniectomy, and applying a square-wave constant-current anodal stimulus. EMG's were recorded in response to this stimulus in the SSM as described above. The time taken from stimulus to the take-off

of the CMAP was taken as the central conduction time and represents the sum of the time taken for upper and lower motor neurone conduction, synaptic delay and muscle activation.

Histology:

Specimens of the C6 plexus root in the posterior triangle of the neck were taken for histological examination. This site represents the anterior primary ramus of C6 and hence would be expected to contain a population of regenerated motor nerve fibres and also a population of intact sensory fibres. These latter remained after dorsal root avulsion because they were distal to the dorsal root ganglion. The specimens were processed as described above to produce 1μm thick resin-embedded transverse sections for light microscopy and morphometric analysis.

Results

Traction Injury Model

All animals were reviewed at 1 year after the nerve-repair operation.

In each animal the specimen of C6 root removed at the time of injury and repair was seen on histological examination to consist of connective tissue with no visibly intact neural tissue. This was taken to indicate that the injury was morphologically consistent with a complete Sunderland type IV lesion.

Age of Recipient (Table 1)

When the repaired groups of sheep and lambs were compared with normal controls in respect of the morphological indices: {fibre diameter, axon diameter, myelin sheath thickness and G-ratio (axon diameter ÷ fibre diameter)}, significant differences were found for the first three but not for the last. This means that although after repair, axon

and fibre size and myelination did not attain normal levels, the degree of myelination was nevertheless appropriate for the size of the regenerated axons. This is in accord with previous work in adult animals (Fullarton, Glasby, & Lawson 1998; Gattuso, Davies, Glasby, Gschmeissner, & Huang 1988; Gattuso, Glasby, & Gschmeissner 1988; Glasby et al. 1990; Glasby, Carrick, & Hems 1992; Glasby, Fullarton, & Lawson 1997; Glasby & Hems 1995; Glasby, Hems, & Pell 1992; Glasby, Mountain, & Murray 1993; Hems & Glasby 1992).

Where the above indices were compared for the groups of lambs and sheep which had undergone excision of the scarred area and immediate repair, there was found to be no significant difference (Table 1, Fig 1).

	Fibre Diameter (μm)	Axon Diameter (μm)	Myelin Thickness (μm)	G-ratio	CMAP Amplitude (V)	CMAP Area (Vs)
CONTROL	16.1 ± 1.3	8.4 ± 0.6	3.9 ± 0.7	0.5 ± 0.1	9.1 ± 4.0	25.6 ± 12.7
SHEEP	8.8 ± 1.7	4.8 ± 1.2	2.0 ± 0.3	0.5 ± 0.0	11.9 ± 3.5	29.0 ± 15.2
LAMB	9.4 ± 0.7	5.1 ± 0.4	2.2 ± 0.2	0.5 ± 0.0	15.8 ± 1.1	40.4 ± 1.2
	CV_{max} ($m\ s^{-1}$)	CV_{min} ($m\ s^{-1}$)	CV_{range} ($m\ s^{-1}$)	*ARP* (ms)	*RRP* (ms)	*TSJ* μs
CONTROL	76.9 ± 7.5	15.7 ± 4.8	61.2 ± 10.3	1.1 ± 0.1	4.8 ± 1.1	13.4 ± 2.6
SHEEP	46.5 ± 7.1	9.8 ± 3.3	36.7 ± 8.7	2.1 ± 1.3	7.2 ± 1.0	17.4 ± 2.7
LAMB	62.0 ± 2.2	13.2 ± 1.2	48.9 ± 3.4	1.2 ± 0.1	6.4 ± 0.5	13.9 ± 1.1

Table 1: Effect of the age of the recipient.

The mean ± standard error of the mean of the experimentally determined values of the electrophysiological and morphometric indices of nerve function in the three groups, control, sheep and lambs.

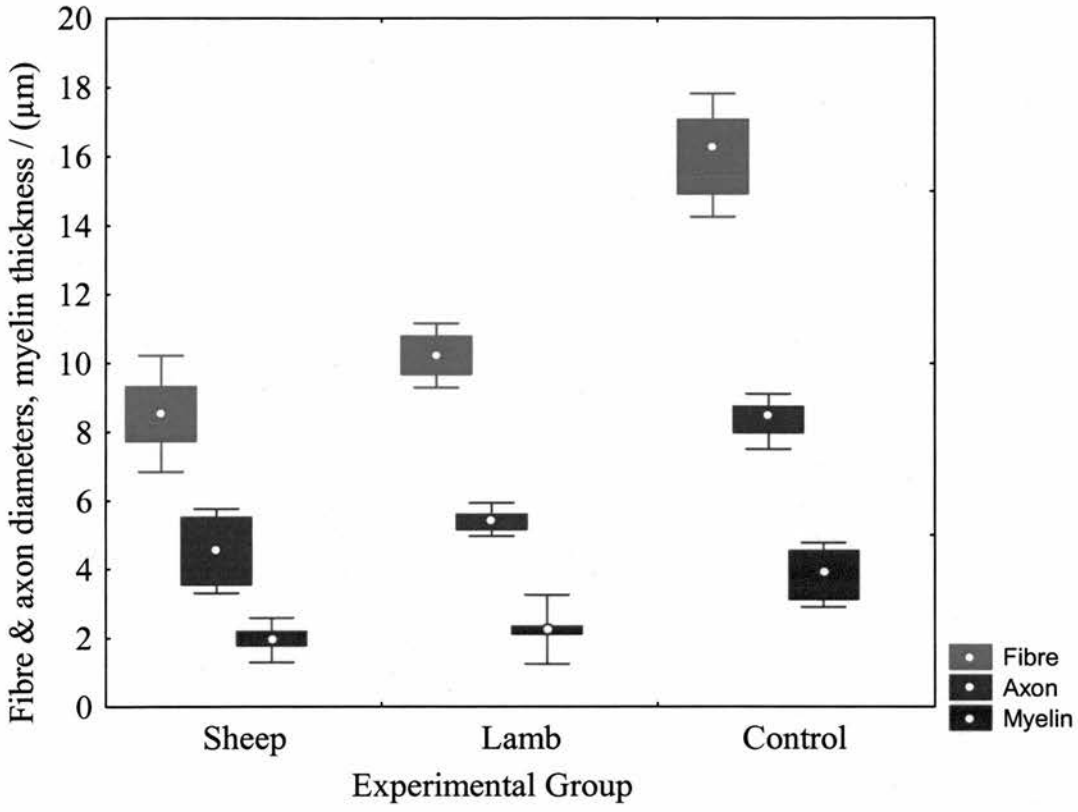


Figure 1

Mean ± standard error of mean (box) and standard deviation (whisker) values of the morphometric indices of recovery recorded in intact 1-year-old sheep and in sheep and lambs 1 year after division and immediate repair of the C6 root of the brachial plexus by means of interfascicular nerve autografts.

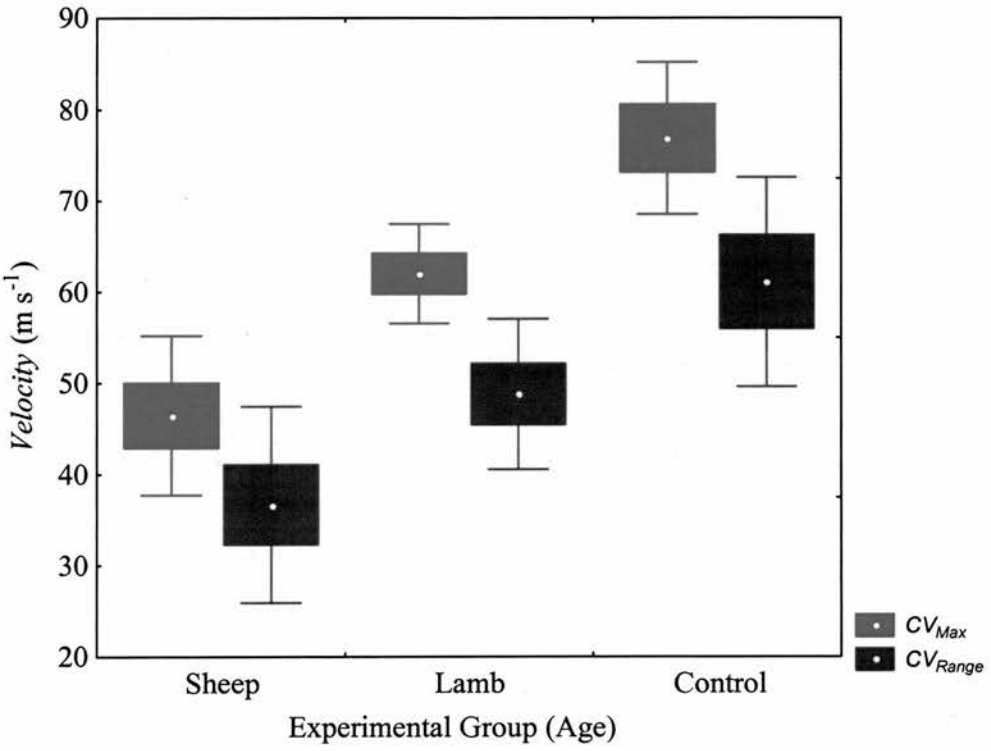


Figure 2

Mean ± standard error of mean (box) and standard deviation (whisker) values of the CV_{max} and CV_{Range} recorded in intact 1-year-old sheep and in sheep and lambs 1 year after division and immediate repair of the C6 root of the brachial plexus by means of interfascicular nerve autografts.

For the electrophysiological studies, a significant difference was found for maximum conduction velocity (CV_{max}) in both groups with neither lambs nor sheep attaining control values: this is in accord with the seminal work of Cragg and Thomas (Cragg & Thomas 1961; Cragg & Thomas 1964).

When the sheep and lambs that had undergone repair were compared with one another with respect to CV_{max} it was found that the lamb group had a mean CV_{max} of 62.0 m s^{-1} whereas the mean for the sheep group was 46.5 m/s: this diffrence was significant ($p = 0.004$).

Although the range of conduction velocities ($CV_{range} = CV_{max} - CV_{min}$) was found to be smaller when both operated groups were compared with controls, the difference was only significant for the sheep ($p = 0.005$). When sheep were compared with lambs, there was no significant difference (Fig 2). This reflects the fact that the sheep never regained the faster velocities which were seen to have recovered in the lambs.

For the remaining variables that were measured, no significant differences were found although in the case of jitter, lamb and control values were very similar at 13.8 μs and 13.4 μs respectively whereas the value for sheep was 17.4 μs. This difference approached significance ($p = 0.07$).

Method of Repair (Tables 2 & 3)

There were no early or late complications of surgery. Morphological examination of the excised segment in each case verified that the injury was that of the model and thus represented a Sunderland Type IV lesion.

When the effects of repair with either cable grafts or muscle grafts in sheep were compared with normal controls it was not surprising to find that repair by both methods was associated with significantly poorer fibre diameters, axon diameters, myelin sheath thickness and CV_{max}.

When the operated lambs were compared with controls a worse outcome was detected for these variables. There was no significant difference to be found when operated and intact groups of lambs were compared for jitter and G-ratio. This suggests that despite the failure to achieve normal indices of nerve morphology and function there was, nevertheless, good maturation of both the extramuscular nerve fibres and the intramuscular nerve endings and end plates.

These findings in the lambs were in contrast to those seen in the sheep where there was a bigger mean value for jitter in the FTMG group (Figure 3). This was significantly different from controls. There was no corresponding significant difference in the jitter measured in sheep when cable-grafted sheep were compared with controls. This suggests that in adults there was poorer maturation of the neuromuscular junctions in the group where repair had been carried out with FTMGs.

When the methods of repair using either type of graft were compared it was found that the only significant differences in sheep were in G-ratio and in jitter both of which were significantly poorer in the FTMG group. No such difference was detected in lambs where there was found to be equal performance for both types of graft in respect of all of the variables which were measured.

	Fibre Diameter (μm)	Axon Diameter (μm)	Myelin Thickness (μm)	G-ratio
Intact	16.08 ± 0.66	8.37 ± 0.28	3.86 ± 0.37	0.52 ± 0.03
CG	8.83 ± 0.86	4.79 ± 0.62	2.02 ± 0.14	0.54 ± 0.02
MG	10.36 ± 1.15	4.86 ± 0.61	2.75 ± 0.31	0.47 ± 0.02
Intact *v* CG	P < 0.001	P < 0.001	P < 0.001	N.S.
Intact *v* MG	P < 0.005	P < 0.001	P < 0.05	N.S.
CG *v* MG	N.S.	N.S.	N.S.	P < 0.05
	CV_{max} ($m\ s^{-1}$)	CV_{min} ($m\ s^{-1}$)	CV_{range} ($m\ s^{-1}$)	*TSJ* μs
Intact	76.92 ± 3.37	15.77 ± 2.38	61.15 ± 5.14	15.00 (12.00–18.00)
CG	46.53 ± 3.56	9.80 ± 1.65	36.74 ± 4.39	18.05 (16.62–19.31)
MG	469.16 ± 1.26	11.17 ± 1.16	34.99 ± 1.95	23.90 (21.92–27.19)
Intact *v* CG	P < 0.001	N.S.	P < 0.01	P = 0.04
Intact *v* MG	P < 0.001	N.S.	P < 0.001	P = 0.006
CG *v* MG	N.S.	N.S.	N.S.	P = 0.03

Table 2: Effect of the method of repair in adult sheep.

The mean ± standard error of the mean (median 25 and 75 percentiles for TSJ) of the experimentally determined values of the electrophysiological and morphometric indices of nerve function in intact animals and sheep after cable graft and muscle graft repair of the C6 root. Probability values for the various comparisons were calculated using the Scheffé test where the data were normally distributed and the Mann-Whitney U test for nonparametric data (TSJ).

	Fibre Diameter (μm)	Axon Diameter (μm)	Myelin Thickness (μm)	G-ratio
Intact	16.08 ± 0.66	8.37 ± 0.28	3.86 ± 0.37	0.52 ± 0.03
CG	9.43 ± 0.78	5.14 ± 0.41	2.15 ± 0.20	0.55 ± 0.01
MG	10.27 ± 0.63	5.51 ± 0.40	2.38 ± 0.13	0.53 ± 0.01
Intact *v* CG	P < 0.001	P < 0.001	P < 0.005	N.S.
Intact *v* MG	P < 0.001	P < 0.001	P < 0.005	N.S.
CG *v* MG	N.S.	N.S.	N.S.	N.S.
	CV_{max} ($m\ s^{-1}$)	CV_{min} ($m\ s^{-1}$)	CV_{range} ($m\ s^{-1}$)	*TSJ* μs
Intact	76.92 ± 3.37	15.77 ± 2.38	61.15 ± 5.14	15.00 (12.00–18.00)
CG	62.03 ± 2.23	13.18 ± 1.22	48.85 ± 3.36	14.03 (11.00–16.12)
MG	58.10 ± 1.91	10.79 ± 1.68	47.31 ± 3.16	12.94 (12.81–13.25)
Intact *v* CG	P < 0.01	N.S.	N.S.	N.S.
Intact *v* MG	P < 0.01	N.S.	P < 0.05	N.S.
CG *v* MG	N.S.	N.S.	N.S.	N.S.

Table 3: Effect of the method of repair in lambs.

The mean ± standard error of the mean (median 25 and 75 percentiles for TSJ) of the experimentally determined values of the electrophysiological and morphometric indices of nerve function in intact animals and lambs after cable graft and muscle graft repair of the C6 root. Probability values for the various comparisons were calculated using the Scheffé test where the data were normally distributed and the Mann-Whitney U test for nonparametric data (TSJ).

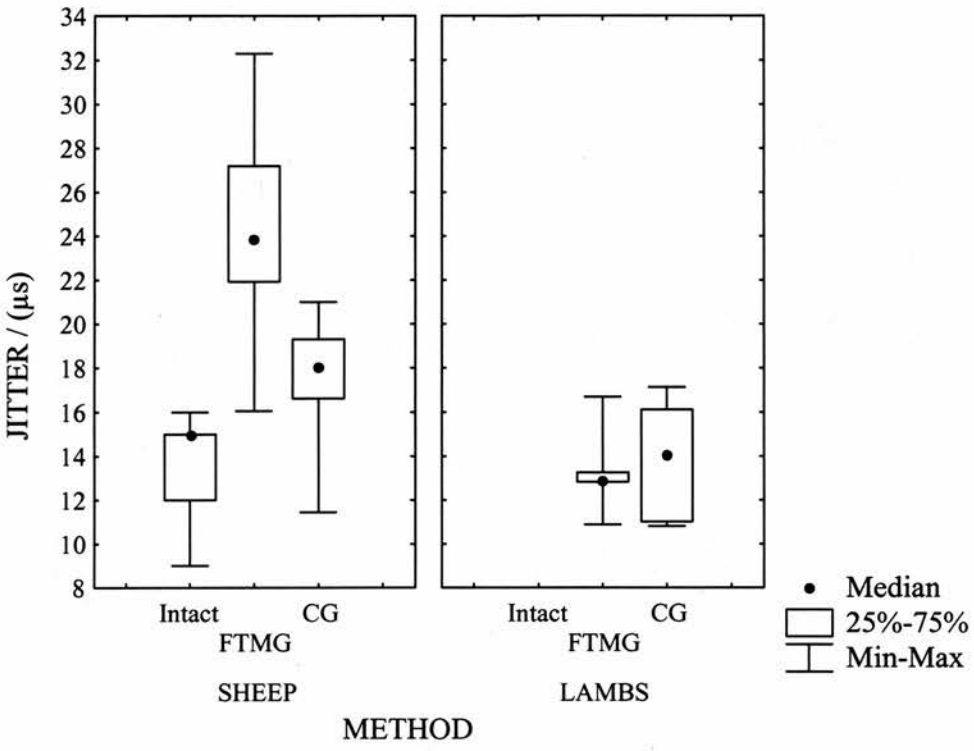


Figure 3

Median ± 25th centiles (box) and maximum and minimum (whisker) values of the *TSJ* recorded in intact 1-year-old sheep and in sheep and lambs 1 year after division and immediate repair of the C6 root of the brachial plexus by means of interfascicular nerve autografts and freeze-thawed muscle autografts.

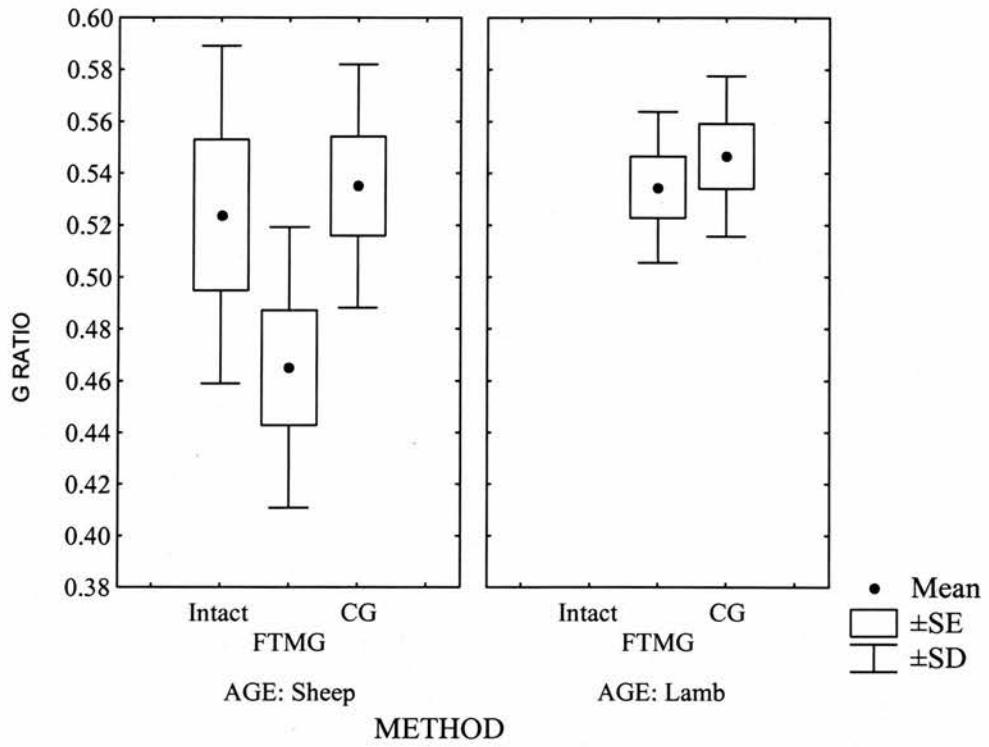


Figure 4

Mean ± standard error of mean (box) and standard deviation (whisker) values of the G-ratio recorded in intact 1-year-old sheep and in sheep and lambs 1 year after division and immediate repair of the C6 root of the brachial plexus by means of interfascicular nerve autografts and freeze-thawed muscle autografts.

Timing of Repair (Tables 4 & 5)

There were no early or late complications of surgery. Morphological examination of the excised segment in each case verified that the injury was that of the model and thus represented a Sunderland Type IV lesion.

When lambs who had received cable grafts immediately after injury were compared with those in which the Sunderland Type IV lesion had been resected and grafted with a cable graft at one month, it was found that there were no significant differences to be detected for any of the variables which were measured. It therefore appeared that after repair using cable grafts, delay for up to a month was compatible with an acceptable level of recovery both of motor function and nerve morphology.

This contrasts with the findings after repair using freeze-thawed muscle autografts where significant differences were found for G-ratio and jitter where the late repaired group showed poorer levels of recovery (Table 5). This means that after repair with the non-neural graft, maturation both of the nerve fibres within the trunk and the intramuscular nerve terminals and end plates was poorer after a delay of one month from injury to repair.

	Fibre Diameter (μm)	***Axon Diameter* (μm)**	**Myelin Thickness (μm)**	**G-ratio**
Early	**9.43 ± 0.78**	**5.14 ± 0.41**	**2.15 ± 0.20**	**0.55 ± 0.01**
Late	**9.73 ± 0.80**	**5.09 ± 0.44**	**2.32 ± 0.20**	**0.52 ± 0.01**
Early *v* late	**N.S.**	**N.S.**	**N.S.**	**N.S.**
	CV_{max} ($m\ s^{-1}$)	**CV_{min} ($m\ s^{-1}$)**	**CV_{range} ($m\ s^{-1}$)**	***TSJ* μs**
Early	**62.03 ± 2.23**	**13.18 ± 1.22**	**48.85 ± 3.36**	**14.03 (11.00–16.12)**
Late	**58.55 ± 2.97**	**12.43 ± 2.08**	**46.12 ± 2.16**	**13.25 (13.13–13.75)**
Early *v* late	**N.S.**	**N.S.**	**N.S.**	**N.S.**

Table 4: Effect of the timing of repair in lambs by means of cable grafts.

The mean ± standard error of the mean (median 25 and 75 percentiles for TSJ) of the experimentally determined values of the electrophysiological and morphometric indices of nerve function in lambs after early and late repair of the C6 root using cable grafts. Probability values for the various comparisons were calculated using the Scheffé test where the data were normally distributed and the Mann-Whitney U test for nonparametric data (TSJ).

	Fibre Diameter (μm)	***Axon Diameter* (μm)**	**Myelin Thickness (μm)**	**G-ratio**
Early	**10.28 ± 0.62**	**5.51 ± 0.40**	**2.38 ± 0.13**	**0.53 ± 0.01**
Late	**10.77 ± 0.60**	**5.23 ± 0.32**	**2.77 ± 0.18**	**0.49 ± 0.02**
Early *v* late	**N.S.**	**N.S.**	**N.S.**	**$P < 0.05$**
	CV_{max} ($m\ s^{-1}$)	**CV_{min} ($m\ s^{-1}$)**	**CV_{range} ($m\ s^{-1}$)**	***TSJ* μs**
Early	**58.10 ± 1.91**	**10.79 ± 1.68**	**47.31 ± 3.16**	**12.94 (12.81–13.25)**
Late	**51.80 ± 2.48**	**10.77 ± 0.60**	**41.03 ± 2.89**	**16.92 (16.06–19.06)**
Early *v* late	**N.S.**	**N.S.**	**N.S.**	**N.S.**

Table 5: Effect of the timing of repair in lambs by means of freeze-thawed autologous muscle grafts.

The mean ± standard error of the mean (median 25 and 75 percentiles for TSJ) of the experimentally determined values of the electrophysiological and morphometric indices of nerve function in lambs after early and late repair of the C6 root using freeze-thawed muscle grafts. Probability values for the various comparisons were calculated using the Scheffé test where the data were normally distributed and the Mann-Whitney U test for nonparametric data (TSJ).

It is helpful in referring the above to clinical situations to obtain an idea of how the three independent variables which were considered in the three parts of this study may interact. The statistical technique of ANOVA was therefore used to identify where differences lay and to assess the 'p-values' that these differences were due to *between-groups-variation* and not to *within-groups-variation*. Applying the ANOVA test to all of the data collected in the study for the three independent variables: age of recipient, type of graft and timing of the repair identified statistical differences in three of the dependent variables, namely: G-ratio ($p<0.05$), CV_{max} ($p<0.001$) and jitter ($p<0.0001$). This is encouraging because the latter two which are the best discriminators are both easily measured in the clinical situation (Figures 5 & 6).

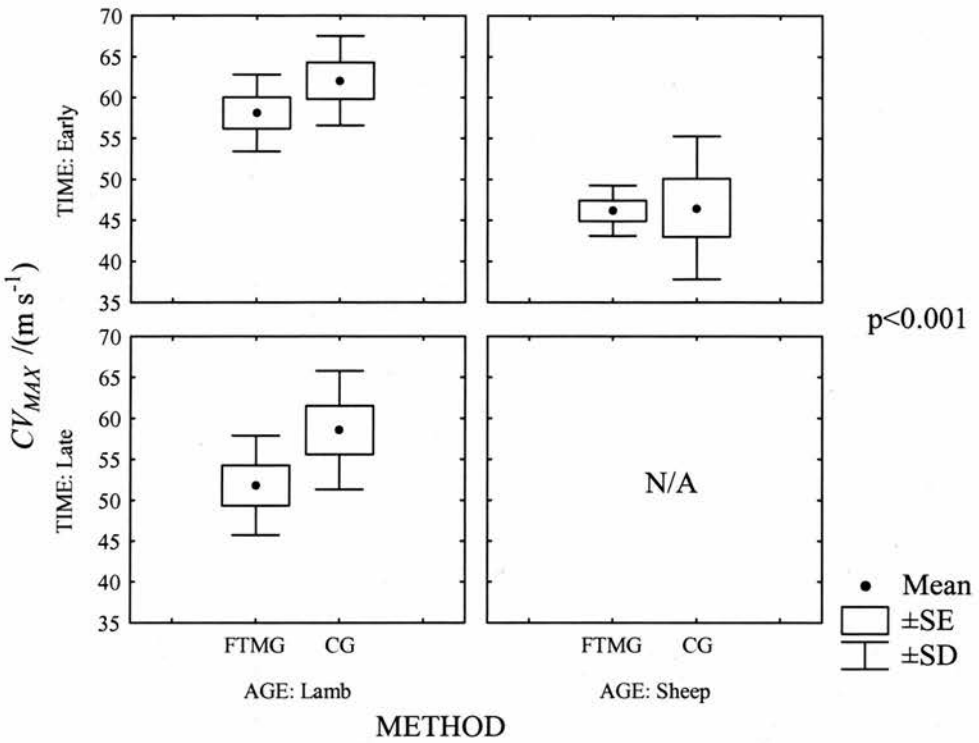


Figure 5

Graphs of the overall effect of the three independent variables ***age*** of animal, ***method*** of repair and ***timing*** of repair for the dependent variable $CV_{max.}$ compared by means of factorial **ANOVA.**

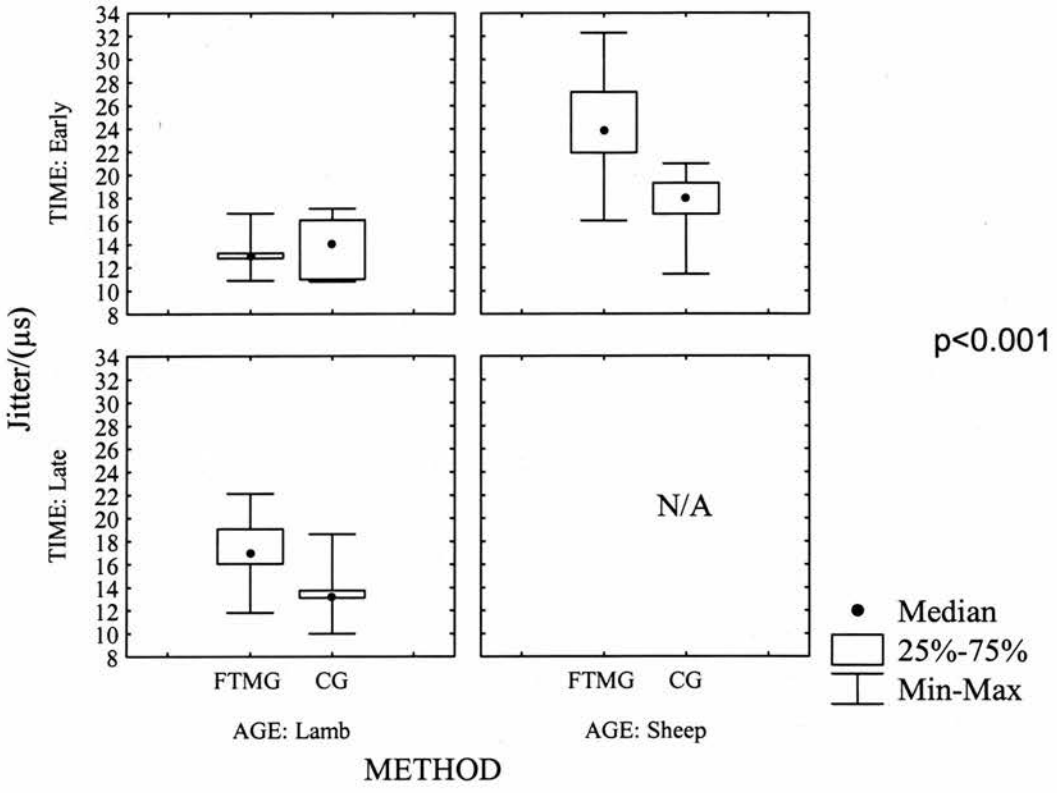


Figure 6

Graphs of the overall effect of the three independent variables ***age*** of animal, ***method*** of repair and ***timing*** of repair for the dependent variable ***TSJ*** compared by means of factorial **ANOVA.**

Avulsion Injury Model

There were no early or late complications of surgery. All animals were reviewed at one year after the root avulsion and repair operation.

When the repaired groups of sheep and lambs were compared in respect of the morphological indices, fibre diameter, axon diameter, myelin sheath thickness and G-ratio, significant differences ($p<0.05$) were found for the first three of these but not for the last. The group that had been repaired as lambs had significantly greater axon and fibre diameters than the group repaired at one year ($p<0.05$). It has been suggested that normal fibre size depends on the attainment of functional end organ connections, which might imply that the lamb group had made more functional connections. G-ratio was not significantly different in any comparison indicating that the axons were appropriately myelinated for their size. As expected, axon and fibre diameters were significantly different in both of the operated groups when compared with normal controls ($p<0.05$). This is particularly well seen in the distribution plots of axon and fibre diameters.

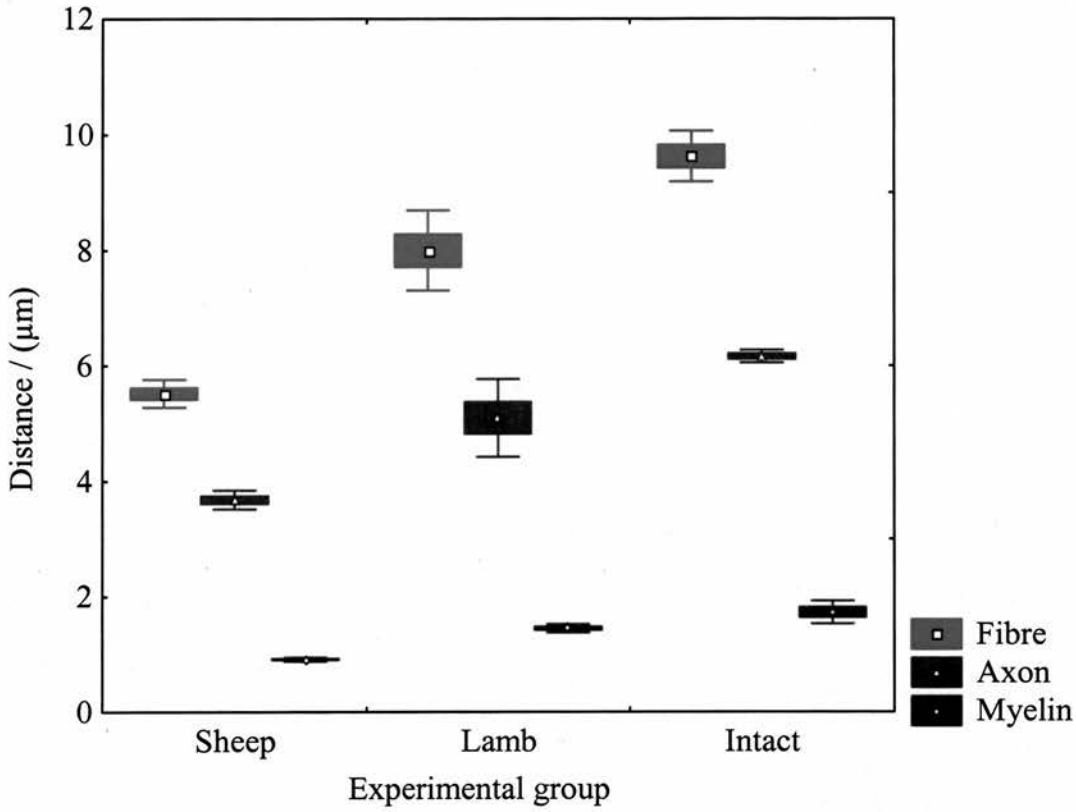


Figure 7

Mean ± standard error of mean (box) and standard deviation (whisker) values of the fibre diameters, axon diameters and myelin sheath thickness recorded in intact 1-year-old sheep and in sheep and lambs 1 year after avulsion and reimplantation of the C6 root of the brachial plexus.

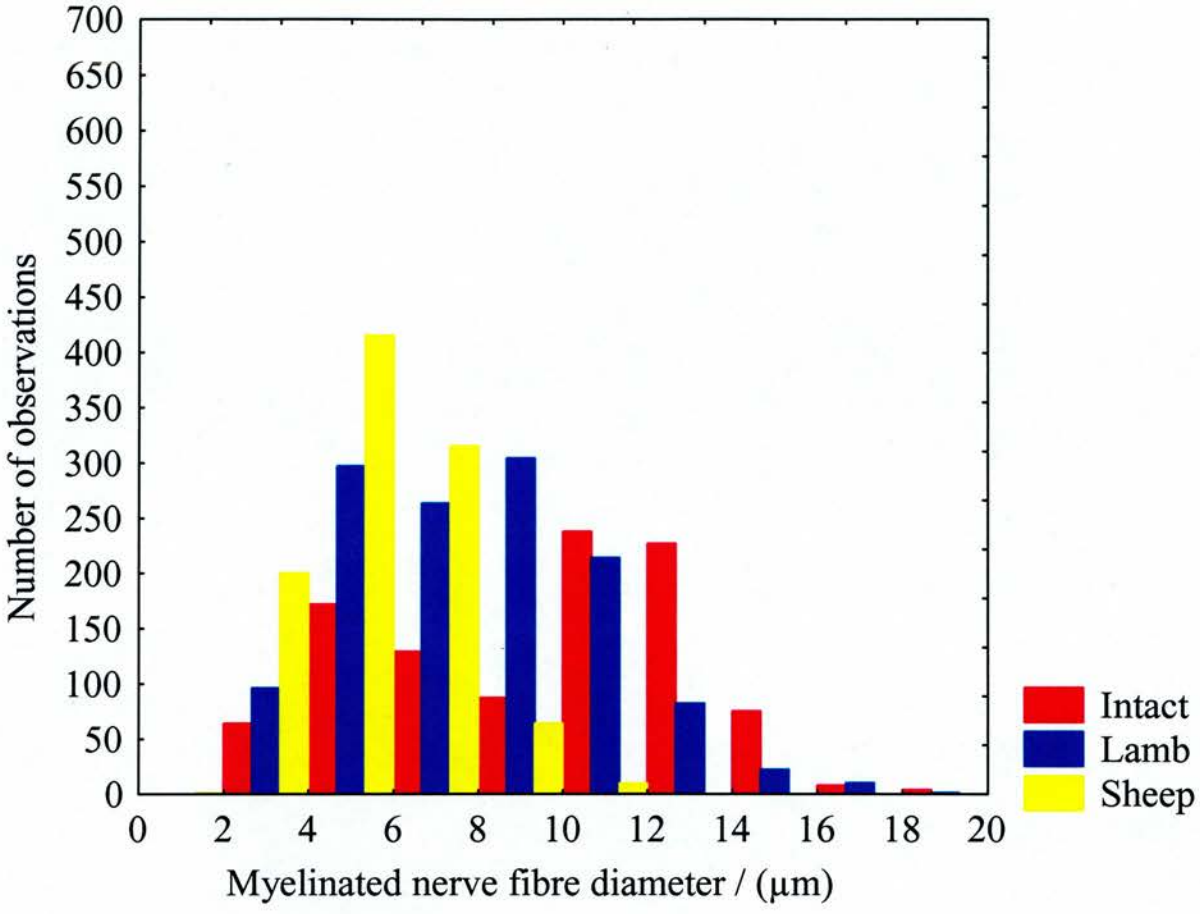


Figure 8

A distribution histogram of the frequency of nerve fibre diameters recorded in intact 1-year-old sheep and in sheep and lambs 1 year after avulsion and reimplantation of the C6 root of the brachial plexus.

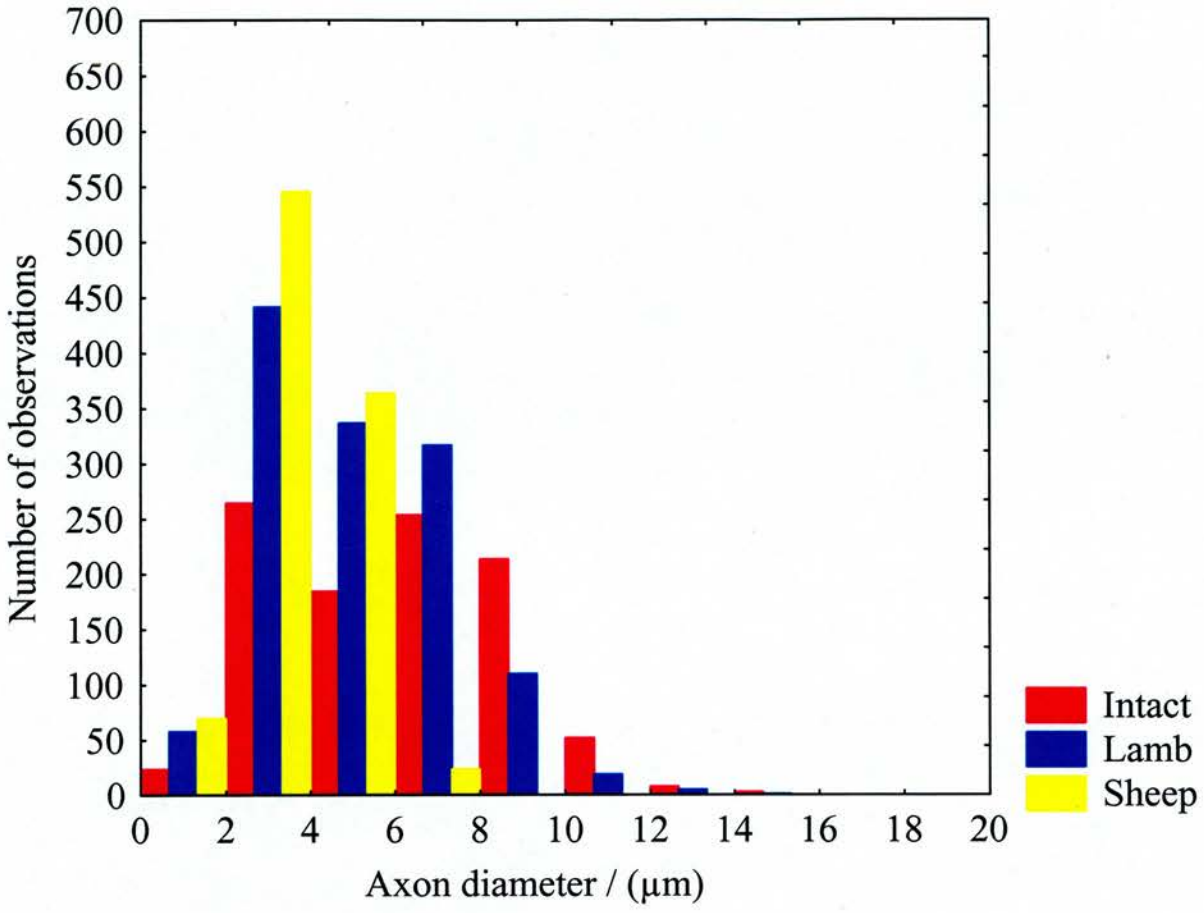


Figure 9

A distribution histogram of the frequency of axon diameters recorded in intact 1-year-old sheep and in sheep and lambs 1 year after avulsion and reimplantation of the C6 root of the brachial plexus.

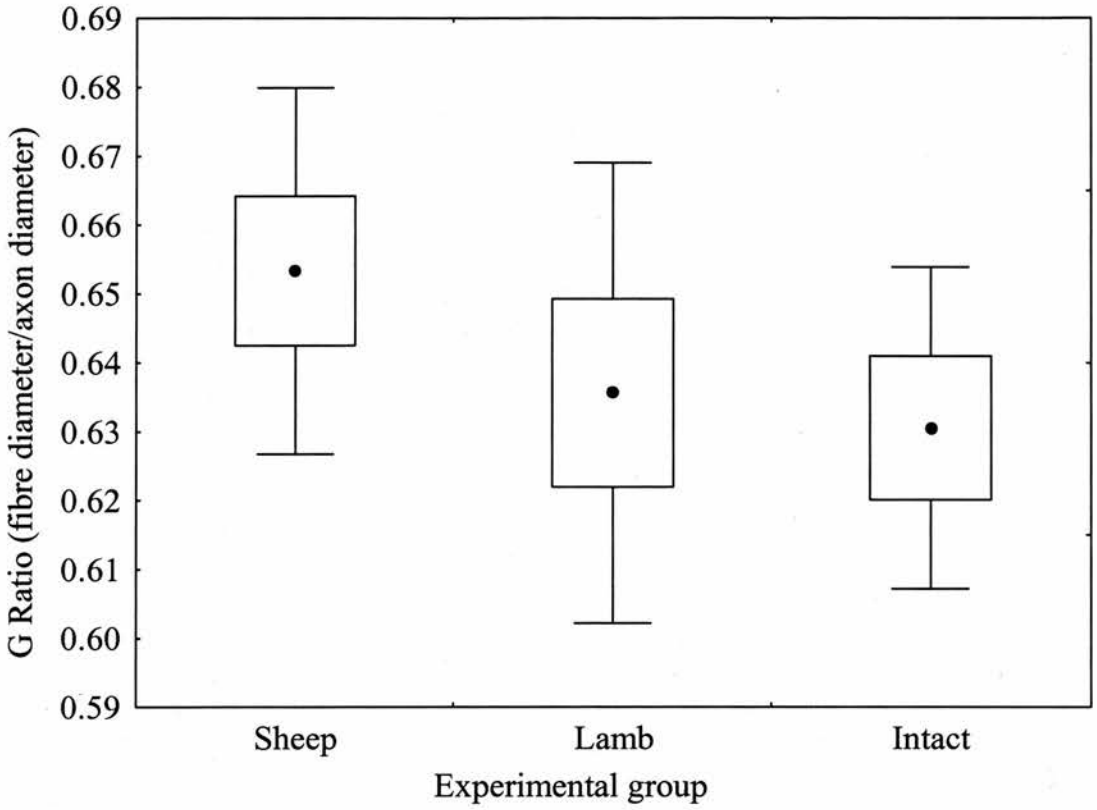


Figure 10

Mean ± standard error of mean (box) and standard deviation (whisker) values of G ratio recorded in intact 1-year-old sheep and in sheep and lambs 1 year after avulsion and reimplantation of the C6 root of the brachial plexus.

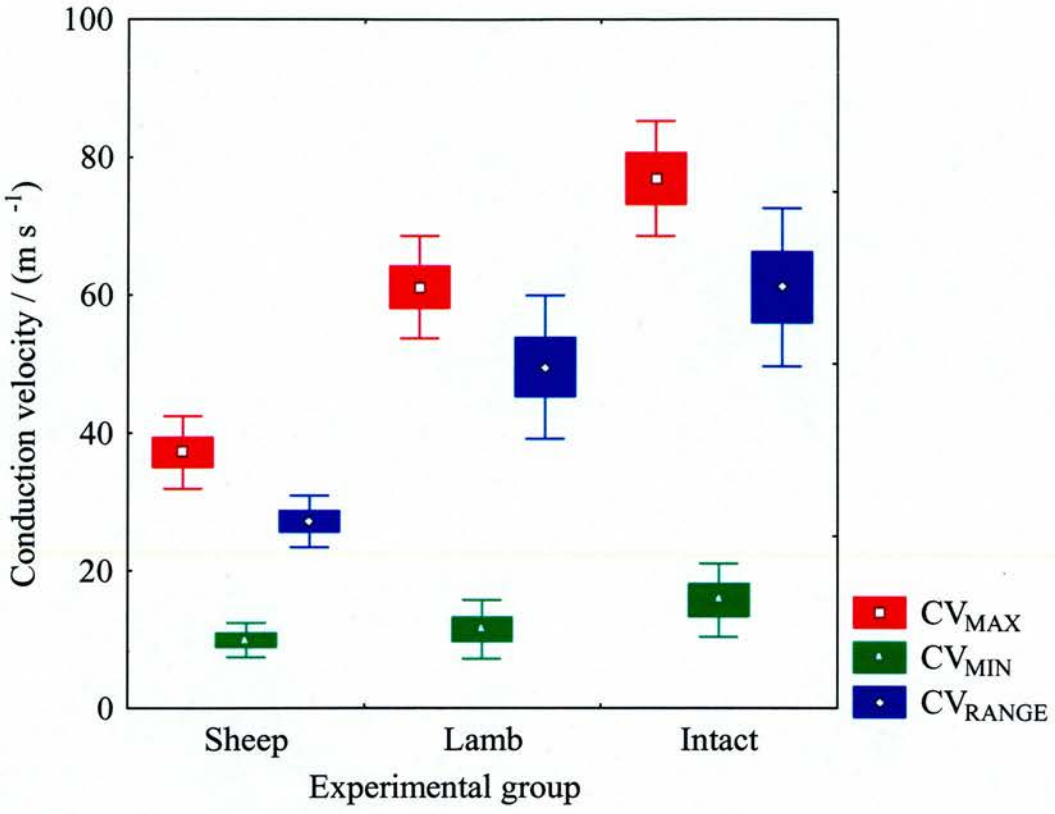


Figure 11

Mean ± standard error of mean (box) and standard deviation (whisker) values of CV_{max} recorded in intact 1-year-old sheep and in sheep and lambs 1 year after avulsion and reimplantation of the C6 root of the brachial plexus.

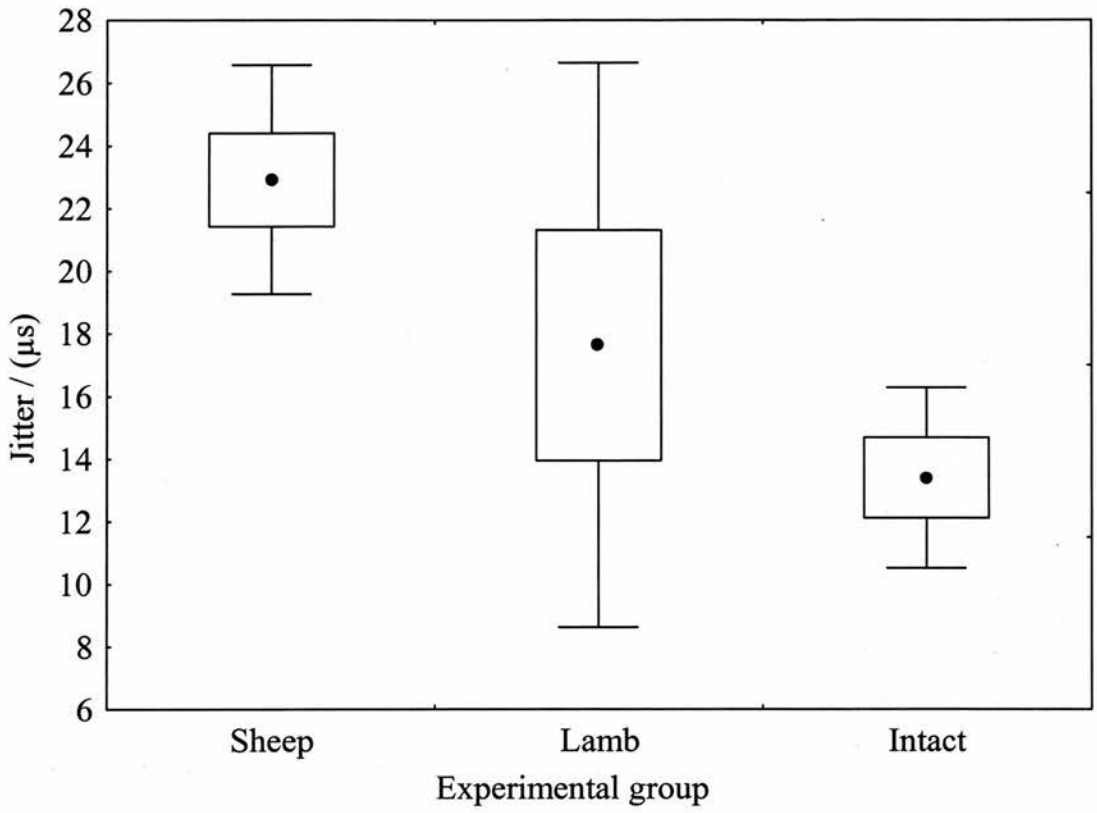


Figure 12

Mean ± standard error of mean (box) and standard deviation (whisker) values of transcutaneous stimulated jitter recorded in intact 1-year-old sheep and in sheep and lambs 1 year after avulsion and reimplantation of the C6 root of the brachial plexus.

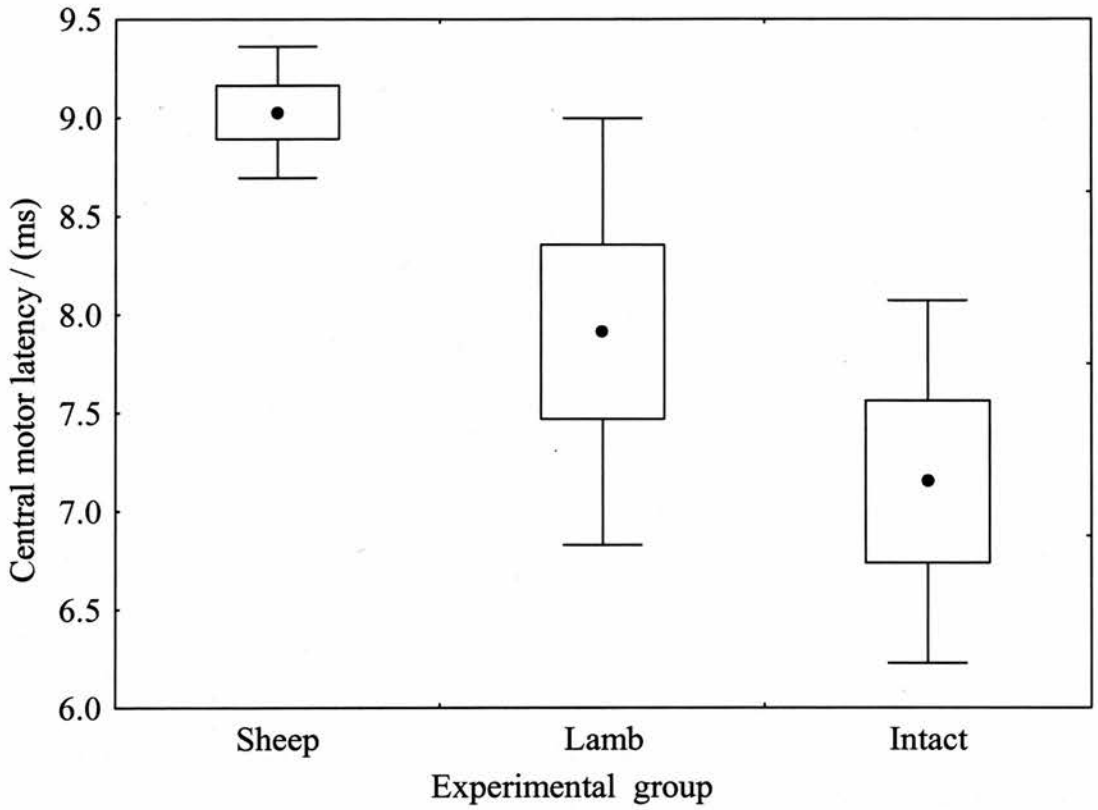


Figure 13

Mean ± standard error of mean (box) and standard deviation (whisker) values of central motor latency recorded in intact 1-year-old sheep and in sheep and lambs 1 year after avulsion and reimplantation of the C6 root of the brachial plexus.

In the electrophysiological studies, a significant difference was found for maximum conduction velocity (CV_{max}) and central motor latency (CML). In the lambs the mean CV_{max} was 61.09 $\pm$ 3.04 m s^{-1} compared with 37.15 $\pm$ 2.16 m s^{-1} in the sheep (p=0.000076). This reflects the greater size of axons seen in the group repaired as lambs. When compared to the normal side (CV_{max} = 61.15 $\pm$ 3.40 m s^{-1}) CV_{max} in the lambs was not significantly different (p = 0.113). The CV_{max} in the sheep group, however, *was* significantly different when compared to the normal group (p = 0.000005)

The range of conduction velocities (CV_{range} = CV_{max} – CV_{min} as CV_{max}— Figure 11) was found to be larger in the group operated on as lambs when compared to the sheep group (CV_{range} lambs = 49.58 $\pm$ 4.24 m s^{-1} as against CV_{range} sheep = 27.18 $\pm$ 1.53 m s^{-1}). This reflects the fact that the sheep never regained the faster velocities that were seen in the lamb group.

Jitter (TSJ) is a sensitive method of assessing the degree of reinnervation at the neuromuscular junction. It is initially prolonged and returns towards normal as reinnervation proceeds. There were no significant differences between the unoperated, control sheep (13.4 $\pm$ 1.18 μs) and the lambs (17.6 $\pm$ 3.68 μs) p = 0.34 (jitter). The sheep group was significantly different from normal controls (22.91 $\pm$ 1.49 μs) p = 0.001. However when the lamb group was compared to the sheep group there was no significant difference (p = 0.212) although the mean value for the lambs was less. This may reflect sample sizes. TSJ was the only electrophysiological variable measured that was not found to be statistically different when the lamb and sheep groups were compared.

No significant difference was found in central motor conduction time (CML) in the lambs (7.91 $\pm$ 0.44 ms) when compared to the control group (7.15 $\pm$ 0.29 ms) (p =

0.245). The difference between the sheep group (9.02 $\pm$ 0.13 ms) and the control group (7.15 $\pm$ 0.29 ms) was statistically significant ($p = 0.001$). The difference between the lamb group (7.91 $\pm$ 0.44 ms) and the sheep group (9.02 $\pm$ 0.13 ms) was also statistically significant ($p = 0.036$).

Conclusions

The traction injury experiments show that the G-ratio measurements in the operated sheep and in lambs were not significantly different from controls. Full maturation of nerve fibres had therefore been attained in all groups and the observed reduction in conduction velocity seen in the operated groups was thus not the result of incomplete maturation. Axon and fibre diameters were found not to be significantly different when sheep were compared with lambs. The smaller CV_{range} and CV_{max} (but not CV_{min}) seen in the repaired sheep group can therefore be explained by a selective failure of regeneration of the largest diameter fibres.

The loss of fast-conducting fibres has traditionally been blamed for the failure of the patient, after nerve repair, to perform fine movements. This is an oversimplified view which has been put down to the fact that fast fibres mediate fine and rapid movements. It seems more likely that the failure is due to the fact that the entire proprioceptive pathway, involving type Ia, Ib and group II fibres on the afferent side and the process of α/γ coactivation on the efferent side, is lost (Carrick, Fullarton, & Glasby 1992). For proprioception to recover, two neural pathways must have been reconstituted and this is most unlikely. In the present study, in respect of CV_{max}, lambs were found to attain significantly faster conduction velocities than sheep. This can only be explained by a greater potential for the regeneration of ***larger*** fibres in lambs than in adult sheep. Why this should be so remains unclear but the clinical

implications need to be considered. One possibility, is that younger animals have a greater potential generally to ***regenerate*** neural pathways. Thus, in the random system of regenerating nerve fibres, it might be expected that young fibres with a higher potential for regeneration would prevail. The probability of any two-way circuit being successfully repaired is low, but it may be ***less low*** in young animals than in adults. The alternative view is that the end-point is the result of greater central (*i.e.* cortical) neuronal ***plasticity*** which compensates for the defective peripheral re-wiring. It is equally possible that the observed results may be due to either or both of these factors.

In practical terms, the surgeon faced with the management of OBPP is not interested in the events which occur in adults. Nevertheless, it is important to know that the regenerative properties of nerves in the newborn are quite different from those in adults; particularly as there is much more information about the latter. The experiments described here may go some way to clarify this problem. The principal finding in the experiments which are presented here was that maturation both of the extra-muscular nerve and of the intramuscular nerve-endings and motor end plates ***in adults*** were affected by the type of graft used for the repair of the nerve trunk. On the other hand neonatal lambs, in which repair was carried out using FTMGs, did not show any differences when compared with lambs whose nerve roots had been repaired with cable grafts. This implies that, within the context of obstetric brachial plexus palsy and its repair, either type of graft (and therefore grafts in general) could, in theory, be expected to provide an equally acceptable result.

From the present results it would appear that FTMGs are equally effective in neonatal lambs as a means of repairing traction injuries of brachial plexus roots. A strategy advocating their use would, in theory, spare the patient and surgeon from the

problems of prolonged operating times, excessive scarring and blood loss. However in the adult it is well established that FTMGs are less supportive of nerve regeneration than nerve autografts and this may temper enthusiasm for a technique which may still be regarded as experimental. Nevertheless where graft material is in short supply, the FTMG offers an alternative. Where a priority has been established in which roots have to be repaired, it may be appropriate not to be sparing with the available nerve autograft in repairing the important roots but to be content to repair the less important roots with muscle grafts.

A number of factors will be important in the surgeon's consideration of whether early or late repair should be carried out. Davis et al (Davis, Martin, & Perret 1947) suggested that delay in repair would lead to 'constriction of functional elements by surrounding scar tissue' and thus advocated early repair to avoid this. Mackinnon and Dellon (MacKinnon & Dellon 1988a; MacKinnon & Dellon 1988b) have found that it is easier to separate the planes of tissue by dissection if operation is performed at an earlier stage.

Electrophysiological investigation in the early time after injury has been met with some skepticism (Slooff 1993; Slooff 1995; Slooff & Blauuw 1995; Slooff & Ubachs 1993). This is because in some patients the lesion is one of neurapraxia manifesting itself as a conduction block which recovers over a relatively short time (Gilbert 1995a; Gilbert 1995b; Gilbert & Tassin 1984; Gilbert & Tassin 1987). Smith, however (Smith 1996) has shown that within this group there exists a subset of cases in which partial rupture has occurred and these patients present as a group which recovers over a longer period than the above *i.e.* beyond four weeks. While this view would support an argument for further expectant treatment Birch and Bonney have made the important point that 'palliative procedures are far inferior to those following

good nerve regeneration' (Birch, Bonney, & Wynn-Parry 1998). Therefore it makes sense to operate on the nerves as early as possible so that if this strategy should fail, there will be less muscle atrophy or skeletal deformity to hamper further reconstructive procedures. In the process of nerve repair itself the other disadvantages of delay which have been demonstrated conclusively are retraction of the nerve stumps, elongation of the scarring (in a Sunderland Type IV lesion) and formation of a neuroma-in-continuity[20]. All of these, after neurolysis and excision *etc.* result in a bigger gap and thus require a longer graft.

From the results presented here it is possible to conclude that delay of up to one month is not associated with any deleterious effects if the conventional strategy of using interfascicular/cable grafts is employed. This means that maximum benefit can be gained from the use of electrophysiological techniques without the concern that an important phase of potential regeneration has been missed. These results do not tell us how long one can afford to wait after four weeks and a further set of studies along the same lines at three months would seem appropriate. What little is known about the maturation of sheep nerves suggests that this process may take place more quickly than in human neonates and infants (Hakamada, Kumagai, Watanabe, Koike, Hara, & Miyazaki 1982) and thus the results presented here may be viewed as giving a favourable understatement of the true situation.

Where there is a shortage of graft material and recourse is made to non-neural autogenous tissue, it appears from the findings here that the results which follow delay are worse and that diagnosis and repair before one month has elapsed is to be

[20] Which Mackinnon has [curiously] termed 'Sunderland Type VI' injury. This nomenclature is wholly inappropriate and appears to miss the point that Sunderland's classification is based upon the anatomical injury rather than upon the response to injury. It is not, therefore *eiusdem generis.*

preferred. It is likely that these cases will also be those where the injuries are most extensive and where the prognosis is poorest for in these cases the other factors discussed above will be operative.

An important point which emerges from this study is that two tests, measurement of CV_{max} and of *TSJ*, both of which are relatively non-invasive and appropriate for outpatient use, offer the best means of quantifying recovery. Moreover since one of these tests is concerned with the nerve trunk and the other with the neuromuscular junction and beyond, the entire lower motoneurone–target muscle complex is assessed. CV_{max} is already the mainstay of conventional nerve conduction studies and although stimulated jitter is more complicated to perform and involves the use of needle electrodes, it is a test well tolerated by most patients in the clinic.

The result of repair in this series was excellent considering the severity of the lesion. This shows that motor axons can regenerate from the spinal cord into the peripheral nervous system and make functioning connections. Carlstedt *et al* have already reported cases where avulsed roots were reimplanted into the spinal cord in adult humans with some success although the technique of repair which they described was slightly different in that they implanted the nerve end directly into the spinal cord (Carlson, Lais, & Dyck 1979; Carlstedt, Linda, Cullheim, & Risling 1986; Carlstedt 1991; Carlstedt & Noren 1995; Carlstedt, Hallin, Hedstrom, & Nilsson-Remahl 1993; Carlstedt 1995; Culheim, Carlstedt, & Risling 1999). In the technique used in the present experiments the spinal cord was not disturbed in any way and the technique was found to be safe and easy to perform using standard microsurgical techniques. The muscle graft acted as a support for the nerve and fibrin glue which otherwise tended to fall away into the subdural space.

Before introducing reimplantation of avulsed roots into widespread clinical practice for OBPP it would be necessary to compare results with those of the current strategy of nerve transfers and take into account the risks of operation. Age may have several effects on the eventual outcome after peripheral nerve repair. It is accepted that after motor nerve section a number of cells die. In the adult this is in the order of 11% – 53% depending on the species and the level of the injury (Arvidsson, Ygge, & Grant 1986). Lesions closer to the cell body lead to more cell death than more peripheral lesions (Carlson, Lais, & Dyck 1979). Neonatal rats experienced greater cell loss than adult rats for a given nerve lesion (Grieve, Kristmundsdottir, & Glasby 1991). However this may be offset by the greater regenerative potential and better functional outcome due to the central reorganization which is often seen in young animals and humans.

There is no doubt that surgery in the new-born is more difficult owing to problems associated with blood loss, temperature regulation and simply by virtue of the smaller size of the operative field. Complications associated with surgery may compromise the eventual outcome and mortality associated with the treatment is unacceptable in a condition which is not normally life- threatening. However if it could be shown that the eventual functional outcome was better if surgery were performed at an early age then the increased risk may be justified.

The present results show a benefit in animals injured and repaired at one week after birth compared with those injured and repaired at one year of age. It would be expected from the evidence discussed above that the animals injured at one week of age would have had a greater degree of cell-death associated with their injury than the older animals. The fact that their eventual outcome was better than the older group, in respect of almost every variable, implies that the regenerative response and/or the

reorganization of the spinal cord was better than in the younger animals. There was no increased morbidity in the lambs despite their smaller size at the time of surgery.

In the present study an attempt was made to provide a model which was as close as possible to the clinical situation and in an animal where nerve size and regenerative rate were not dissimilar to that in humans. This experimental model nevertheless involved an injury made under ideal surgical conditions and uncomplicated by additional pathology. The implications for the use of the technique in clinical practice may be viewed with some enthusiasm based upon the results obtained here but remain to be evaluated in the more complex context of human neurosurgery.

CHAPTER 8 — COMPLICATED NERVE INJURIES

COMPLEX INJURIES IN CLINICAL PRACTICE

THE reality of clinical practice is that most injuries are, to some degree, complicated. By this is meant that few injuries present themselves to the clinician in the precise nature of those injuries embodied in the various classifications of nerve injury. As a result there must always be a degree of compromise in the way in which these injuries are handled. For the laboratory investigator the move from controllable paradigms to models of reality is difficult because most of the criteria built up from studies of exact models will now become blurred. Moreover a new and overwhelming priority — that of preserving life — becomes associated with many of these injuries and management of the nerve injury may be compromised by this absolute need and by the lesser needs to consider other serious, but not life-threatening, injuries before the nerve. Nevertheless in the long term the consequences of serious nerve injuries are the cause of great distress and disablement so it is important that the best and earliest possible reasonable consideration should be given to their management.

Value of laboratory studies

Up to a point, the laboratory provides a means of studying complex injuries inasmuch as models may be created to mimic at least some of the problems seen in multiple trauma. In the present study three such models were compared with the simple injury of the median nerve in the sheep and its repair using a short freeze-thawed muscle autograft (FTMG). The associated injuries which could be created and repaired in association with the nerve injury and its repair were:

- Cavitation, fibrosis and haematoma
- Long bone fracture
- Adjacent injury and repair of a major artery.

It will be noted that the seemingly obvious association of nerve injury with local infection is not represented. This model would be very appropriate to the present study, however the logistics of carrying out such a study with an infected/ious population of animals was not appropriate or possible at EPNRL on account of the the risk to other animals and a Home Office Licence for this procedure was not obtainable — a pity, but understandable.

In each of the above cases it has to be admitted that the model was somewhat contrived but nevertheless an order of magnitude more 'realistic' than most experimental models considered hitherto. The results indicate the value of such experiments by showing that the associated injury, in each case caused overall greater morbidity ***in terms of the outcome of the nerve repair*** than was seen in the case of simple injury. These experiments also provided an opportunity to consider the effect upon outcome of delaying the nerve-repair procedure for a month after the operation to deal with the complicating injury. This is important because delay of the nerve-repair procedure is common where other injuries are in association and may be thought to require more urgent attention.

Timing of repair

The optimal timing for peripheral nerve repair remains a matter of some controversy. The choice, where it exists, lies between immediate repair and delayed (secondary) repair. It was concluded in the 1954 Medical Research Council Report (Medical Research Council 1954) that early secondary repair was the procedure of choice, as it

allowed better recognition of damaged nerve for neurolysis, and as the epineurium would be thicker it would be better able to support sutures. This recommendation was in contradistinction to that of Platt (Platt 1919; Platt 1921) who had made the firm assertion that late repair was the method of choice. This was most probably because of the convenience of dealing with associated injuries rather than as a result of any studies on the recovery of nerve function. However a voice such as Platt's carried great weight and the argument for delaying repair has persisted to an extent which is almost certainly at odds with best practice. There are many who still believe that the case for early repair is not proven. That is not the view of the present author. However it has to be accepted that although the case for early repair is overwhelming in ideal circumstances, such circumstances are rare. The question therefore remains as to what criteria should be applied in deciding how far delay can be employed with the expectation of useful outcome.

There are significant disadvantages in delayed nerve repair. After nerve transection the two ends retract owing to nerve elasticity. In response to trauma, there is a degree of fibrosis both proximal and distal to the site of transection and this results in loss of elasticity. In addition, the formation of adhesions reduces the gliding capacity of the nerve. In order to achieve secondary end-to-end neurorrhaphy, one must overcome not only the elastic retraction of the nerve but also the fibrotic retraction, with more radical neurolysis. Where adhesions have formed, an even distribution of forces along the length of the nerve may not be achieved, resulting in greater tension at the suture line (Millesi, Zoch, & Rath 1990). There may also be a progressive decrease in the diameter of the endoneurial tubules and fascicles (Hammond & Hinsey 1945).

With the development of microsurgical techniques (Khodadad 1972), and a changing spectrum of nerve injuries, primary repair has become the treatment of choice

wherever possible (Birch, Bonney, & Wynn-Parry 1998; Grabb 1968; Sunderland 1978; Sunderland 1991). Currently, delayed nerve repair is indicated in complex cases of severe local soft tissue and/or bony injury associated with a significant area of nerve injury or indeed loss, and a ragged nerve transection (Kline 1990).

Nerve grafts

In addition to life-threatening injuries many of the circumstances requiring delay of the nerve repair may also be associated with the need to insert a graft (Gattuso et al. 1989). The adverse effects of any degree of tension at the site of neurorrhaphy are well recognized (Millesi 1980; Millesi 1991; Millesi & Meissl 1981; Millesi, Meissl, & Berger 1972; Millesi, Meissl, & Berger 1976; Terzis, Faibisoff, & Williams 1975), and the limitations of interposition of nerve autografts are well established (Birch, Bonney, & Wynn-Parry 1998; Seddon 1975). Gattuso et al (1989) looked at delayed repair with FTMG in the rat sciatic nerve and noted that the FTMG was of use but that a delay conferred no advantage over immediate repair. This study, however utilized a simple and ideal small animal surgical model.

Penetrating missile injuries

A further group of injuries where complication of the nerve injury *per se* is almost inevitable is that involving penetrating missile injuries from either bullet, shotgun or shrapnel and can result in a wide range of soft-tissue injuries. Frequently such wounds represent the most severe and dramatic of all soft-tissue injuries and require meticulous debridement. In 1497, one of the first detailed accounts of gunshot wounds and their management was written by an Alsatian army surgeon named Heironymus Brunschwig, who considered all shotgun wounds as poisonous. By 1563, the Elizabethan surgeon Thomas Gale had recognized the need for surgical

debridement and excision of all infected or devitalized tissue (Gray 1915). Since that time, with each successive military conflict, the management of penetrating missile trauma has continued to improve. Advances in patient resuscitation, surgical technique and the use of broad spectrum antibiotics have all resulted in a decreased mortality and morbidity (Omer 1991). One of the most significant determinants of final outcome by which especially is meant long-term outcome however, is the presence of a concomitant nerve injury (Peacock & Proctor 1977; Ruijten et al. 1994; Visser et al. 1980).

Undoubtedly, wound severity and the incidence of associated nerve injury depends upon the population under consideration. In civilian practice worldwide, low velocity hand-gun injuries predominate (Victoroff et al. 1996) although there is an alarming increase in the incidence of shotgun injuries (Stewart & Kinninmonth 1993). The incidence of associated nerve injury in civilian firearms injuries varies from 2.5 to 20%. In the military setting, the incidence of peripheral nerve injuries in all *non fatal* wounds during World War I was 2% (Omer 1991). This rose to 6.6°/, in World War **11** and to 7.3% in the Vietnam war. In the Gulf conflict of 1991, nerve injuries were associated with 481 penetrating wounds. It should be noted, however, that over 90% of penetrating wounds in this review were the result of shrapnel injuries (Bajec, Gang , & Lari 1993).

Cavitation

Penetrating missiles may be classified into two categories. shrapnel/fragments or bullets. In recent military campaigns, the former has caused the bulk of injuries. The interaction of the missile with soft tissues is influenced by its mass, velocity and shape as well as its tendency to pitch, roll and yaw and also by the tissue to which it is directed. The potential of a missile to disrupt is determined by its kinetic energy

($\frac{1}{2}mv^2$), while the proportion of that energy that is actually transferred to the surrounding tissues is determined by the degree of retardation of the projectile by those tissues. This obviously depends on the presented area of the missile and thus the effects of pitch and yaw, as well as the nature of the tissues through which it is passing. The effect of the missile can therefore be described in terms of energy-transfer. In low energy-transfer wounds, soft tissue injury is confined to the track of the projectile as it penetrates the tissues. Most antipersonnel fragments and hand-gun wounds fall into this category. In high energy-transfer wounds, in addition to the mechanical disruption (laceration and crushing) produced directly, indirect injury also occurs radial to the path of the projectile. This is caused principally by the formation of a temporary cavity, a consequence of increasing levels of energy being transferred. It should be noted, however, that cavitation occurs with all penetrating missile injuries although the minor degree of cavitation associated with a low energy transfer wound is probably of only minor pathological significance (Cooper & Ryan 1990).

When such wounds are associated with a nerve injury or transection, it is advocated that damaged (identifiable as non-viable) nerve is resected and the nerve stumps secured to healthy nearby tissue in an attempt to prevent further retraction and to allow reconstruction at a later date. The long-term results of peripheral nerve injury and repair associated with severe soft tissue damage, however, remain inconsistent (Bjorkesten 1947; Omer1974).

Injury to long bones

Although the complicating process in the majority of missile injuries is usually multiplex, long bones are frequently injured. In civilian practice, 80–90% of nerve injuries associated with traumatic fractures occur in the upper limb (Cooper & Ryan

1990; Gurdjian & Smathers 1945; Omer 1974); the radial nerve is most commonly affected.

Over the last 50 years, the management of nerve injuries associated with long-bone fractures has changed dramatically. This has undoubtedly stemmed from a better knowledge of the natural history of such lesions coupled with improved methods of assessment of nerve injuries. It is now established that spontaneous neurological recovery will be observed in up to 80% of all nerve palsies associated with upper limb fractures (Omer 1974; Pollock et al. 1981; Pollock et al. 1981; Siegel 1991). In other words, neurotmesis is rare in injuries of this sort, and earlier reports describing the need and efficacy of early exploration and neurolysis (rarely with neurorrhaphy) are probably less relevant today. In contrast, where the combined bony and nerve injury is caused by a high-velocity projectile the result is more often than not the characteristic cavitation which is produced by the pitch and yaw of the missile and by the pressure wave resulting from the bullet or shrapnel. This cavitation effect frequently results in complete rupture of the nerve with, all too often, loss of length (Cooper & Ryan 1990). As a result, the nerve must be repaired by grafting, and this implies an inevitably poorer final result.

While an 'expectant' policy currently prevails in the management of nerve injuries associated with long-bone fractures, those cases which do not exhibit any spontaneous recovery after 4 to 6 months are then explored. Such delayed repair has a far higher requirement for nerve grafting techniques owing to scarring and fibrotic retraction of the nerve stumps. The question of whether more strenuous combined efforts for the primary repair of both nerve and bone injuries should be made remains to be established. In the present study immediate and delayed nerve reconstruction with

FTMGs adjacent to an osteotomy of the radius in the forelimbs of sheep were performed.

Injury to blood vessels

Where the nerve injury is associated with injury to a major blood vessel it might be expected that the combination of injuries would be particularly serious and associated with a very poor long-term outcome. Improved techniques in repair or grafting of vascular trauma coupled with judicious wound debridement and antibiotic therapy have resulted in an ever increasing rate of limb salvage for these injuries (Omer 1974; Omer 1991). This in turn has, however, disclosed the part played in outcome by the nerve injury which, given successful limb-salvage has become the main determinant of ultimate limb function. In civilian practice, 40–50% of vascular injuries to limbs are associated with nerve damage (Nichols & Lillehei 1988; Smith , Elliott , & Hageman 1974). In these combined neurovascular injuries, the incidence of long-term functional disability is unacceptably high at between 45% and 75% (Peacock & Proctor 1977; Visser, et al 1980). Although there have been some experimental and many clinical studies, the effect that a concomitant vascular injury has on peripheral nerve repair and regeneration remains unclear.

In the pre-war period there was some dispute as to whether or not recovery after a crush injury to a nerve was influenced by ligation of the major vessels in the region. Later Shumacker et al (Shumacker , Boone , & Kunkler 1953) noted that return of function after sciatic nerve crush injury in the cat was delayed when it was associated with ligation of the common iliac or common femoral arteries. Kline et al (Kline, Hayes, & Morse 1964) demonstrated that stripping the extrinsic blood supply over a distance of 12 cm distal to either a crush or a transection injury of a

rhesus monkey's sciatic nerve did not alter regeneration and the outcome of repair. In addition Gilliatt and Wilson (Gilliatt & Wilson 1954) pointed out the deleterious effects of acute ischaemia on nerve function.

Starkweather et al (Starkweather et al. 1978) reported that stripping the mesoneurium for 5 cm on either side of a median nerve transection repaired by subsequent neurorrhaphy in rhesus monkeys, resulted in a poorer outcome than minimal (0.5 cm) mesoneurial stripping. It appears from much of this work that the ischaemic effect exerted on the nerve takes place as a result of the loss of small feeding vessels which themselves become functionless when the major vessel of supply is compromised. These small vessels may themselves be compromised by cavity formation and by fibrosis or local compression as well as by damage to the supplying vessel. Thus in a gunshot wound involving a major vessel causing cavitation is likely to have a very serious effect upon any adjacent nerve.

Much of the clinical information regarding the effect of vascular injury on nerve regeneration has arisen from military conflicts. Several authors (Bjorkesten 1947; Richards 1954; Sunderland 1978; Sunderland 1991) have concluded that a vascular lesion and its subsequent management has little influence on the rate of recovery and final outcome of nerve regeneration. This conclusion was reiterated by Gelberman et al (Gelbermann et al. 1979) who stated that 'Associated unrepaired arterial lacerations have no apparent effect on the rate or completeness of neurologic recovery following repair'.

In contrast there is, however, a body of clinical evidence to suggest that arterial damage is an important factor and that arterial repair, besides its obvious effect upon limb perfusion as a whole, is necessary specifically to optimize nerve regeneration (Birch, Bonney, & Wynn-Parry 1998; Cikrit et al. 1990; Leclercq et al. 1985;

Peacock & Proctor 1977; Seddon 1975) noted that successful arterial repair in combined ulnar artery and ulnar nerve lacerations was associated with improved neurological recovery on clinical examination.

The experiments presented below represent an attempt using past experience of the sheep median nerve model for nerve repair to assess surgical models each consisting of an injury and repair to a major (median) nerve in association with a single complicating injury. In one cohort of animals the nerve repair was carried out at the same operation as that used to create and remedy the associated injury and in the other cohort the repair of the nerve injury had been delayed for 30 days.

METHODS

Experimental groups

Each experimental group contained 5 adult ewes each weighing approximately 60kg.

The groups were designated as follows:

1. Neurotmesis + immediate repair by FTMG
2. Neurotmesis + delayed repair by FTMG
3. Neurotmesis + cavitation, fibrosis and haematoma + immediate repair by FTMG
4. Neurotmesis + cavitation, fibrosis and haematoma + delayed repair by FTMG
5. As group 3 + long bone fracture
6. As group 4 + long bone fracture
7. As group 3 + arterial injury and vein graft
8. As group 4 + arterial injury and vein graft

Delay = 30 days. All animals were assessed at 6 months.

For this study, the median nerve of the upper foreleg was chosen, being a large mixed nerve of similar size to the human ulnar nerve at the wrist. High division of the median nerve in the sheep weakens flexion of the forefoot and wrist but more importantly does not affect limb extension required for weight-bearing and locomotion. In addition, such is the degree of overlap from the ulnar and radial nerves that hoof anaesthesia and sepsis are rarely problems.

Creation of the nerve injury and its repair

Simple injury (Groups 1 & 2)

Anaesthetic and monitoring techniques and the surgical approach to the median nerve in the forearm of the sheep have been described in Chapter 2. In the relatively long operations where a bone or artery was to be repaired it was thought helpful if neuromuscular blockade was used. This allowed better ventilation of the sheep and made tissue handling easier as muscle tone was overcome. In consequence neuromuscular blockade with mivacurium (Mivacron; Wellcome; 200 pg kg^{-1}) was used in all of the first operations to create and repair the injuries. The level of blockade was monitored with a nerve stimulator at the facial nerve. In the assessment experiments neuromuscular blockade was never used.

In each case, the median nerve was identified and a 3 cm segment excised using a Meyer neurotome 3 cm proximal to the origin of the anterior interosseous branch. Thus a similar length of nerve had been resected in all of the animals. However in those receiving late nerve repair a variable degree of stump retraction had taken place so this cohort all needed longer FTMGs than the 'immediate repair' group (1). Although this may be seen as 'non-standardization' of the experiments, the view was taken that this was a more realistic representation of the clinical situation and

would be one of the contributing factors to any difference in outcome between the 'immediate' and 'delayed' cohorts.

For the muscle grafts, a strip of muscle (8 x 2 cm) was harvested through the same incision from the free edge of biceps. The graft was then frozen to thermal equilibrium in liquid nitrogen and thawed in distilled water, during which period it underwent shrinkage of about 33%. The graft was fashioned into a rectangular block, 4 cm to 5 cm long and 0.5 cm in diameter. It was then trimmed to fit the nerve gap without creating tension, interposed in the nerve gap and secured by means of four to six 10/0 mono-filament polypropylene (Prolene; Ethicon UK Ltd.) sutures. The suture lines were sealed with fibrin glue (Tisseel).

The wound was closed in layers with 1/0 and 3/0 Vicryl (Ethicon UK Ltd) with a subcuticular skin closure. The sheep, usually fully mobile within an hour of surgery, were kept in the animal house for between 3 and 7 days. Thereafter they returned to the field station without restriction of activity.

In those sheep undergoing delayed repair (group 2), after a segment of median nerve had been excised, the wound was closed as above. These sheep returned 30 days later for definitive nerve repair with a FTMG as described above.

Cavitation, fibrosis and haematoma (Groups 3 &4)

Three procedures designed to recreate a reproducible cavitation injury were undertaken. Using Mayo cholecystectomy clamps, the exposed superficial muscle and soft tissue around the neurovascular bundle was crushed (to the same point on the ratchet) to produce both bruising and areas of devitalized tissue. After this, all the exposed soft tissues were thoroughly abraded using a neurosurgical "Sonocut Maxi" ultra-sonic dissector (Codman Ltd, Johnson & Johnson Company). Lastly, the exposed tissues were painted with "Gurrs" Beechwood Creosote (George T. Gurr Ltd.

London SW6). This resulted in a widespread inflammatory response of the exposed tissues.

The wound was closed as before (after nerve repair in group 3) and 10 ml of venous blood taken from a peripheral venous cannula was instilled into the depths of the wound cavity via a 16 gauge "Tru-Cut" cannula that had been inserted prior to closure of the wound. This was an attempt to mimic a haematoma. The cannula was finally removed.

In both experimental groups (3& 4) the same technique was used to create the cavity, tissue damage and haematoma. The sheep in group 2 which were returned for definitive repair at 30 days all showed at this time a clearly defined neuroma on the proximal nerve stump and a corresponding glioma on the distal stump of the divided nerve. These were resected back to normal nerve as far as could be seen under the operating micro-scope. The resulting gap was always larger than that of the sheep in group 3 (~5 cm). Grafts of 5 to 7 cm (mean 5.8 cm) were therefore used in group 4.

Long bone fracture (Groups 5 & 6)

The median nerve was divided and repaired in the forearm where it lies adjacent to the radius. In the sheep. the ulna consists of little more than a large olecranon process giving attachment to elbow extensor muscles and a very rudimentary shaft which fuses to the radius for almost its entire length. It is insignificant in bearing weight. At the time of making the nerve injury, soft tissue trauma to produce cavitation, fibrosis and a haematoma was produced in both experimental groups in the manner described before.

The slightly concave inferomedial surface of the radius was exposed and a transverse osteotomy was made with the aid of an air-powered reciprocating saw, at a point adjacent to the median nerve in the proximal half of the bone. The two

bone fragments were seen to be clearly separated. The osteotomy was then repaired using an 8 hole stainless steel dynamic-compression plate (Synthes, UK) in the manner described in the 'AO Manual' (Perren 1991; Schatzker 1991). The wound was closed in layers. Pilot experiments had indicated that there was no requirement to support the repair with a plaster cast, and indeed the sheep did not tolerate these well. The sheep were usually fully mobile within an hour of surgery and able to bear weight on the `fractured' leg at an early stage. They were kept in the animal house for 7 days. Thereafter, they were returned to their farm without restriction of activity.

Arterial injury and repair with a reversed vein autograft

Injury and repair of the brachial artery was carried out as follows. The brachial artery was mobilized adjacent and parallel to the site of the median nerve injury. After giving an intravenous bolus of 3 mg kg^{-1} of heparin, the proximal end of the exposed artery was clamped with a short Castaneda clamp and a DeBakey bulldog clamp was applied to the artery as far distally as was possible. A 5 cm length of artery was excised between the clamps. Adventitia was circumcised from the cut ends of the artery which were then flushed out with heparinized saline. The excised arterial segment was replaced with a vein autograft taken from one of the superficial veins accessible through the same incision. Any side branches of the vein graft were ligated flush with the main vessel wall, using 6/0 braided polypropylene (Ethibond, Ethicon UK). The vein segment was flushed out with heparinized saline at low pressure and then reversed about its long axis for insertion into the arterial defect. Anastomosis was facilitated by using a framed Acland microvascular anastomosis clamp and an operating microscope to assist vision. Stay sutures of 9/0 monofilament polypropylene (Prolene, Ethicon UK) were placed at 120° intervals around the

circumference to appose the vessels and the anastomoses were completed with interrupted sutures of the same material: the stay sutures were tied last. When the anastomoses were thus completed the distal and then the proximal clamps were released and gentle pressure maintained until any minor bleeding had stopped. Major bleeding points, if present, were controlled with further sutures. The presence of blood flow in the graft was ascertained by the compression-release test using gentle compression with two sterile cotton wool "Q-Tips". Thereafter, through-out the remainder of the procedure, periodic checks were made for adequate pulsatile blood flow.

Assessment

Six months after nerve repair, each sheep was anaesthetized and the grafted nerve exposed. The contralateral (left) median nerve was also exposed to act as a control against which the grafted nerve could be compared.

The test which were carried out were used to assess the dependent variables:

1. CV_{max}
2. Nerve blood flow
3. Nerve morphometry, (Axon and fibre diameters, myelin sheath thickness).

CV_{max}

The technique for standard nerve conduction studies has been described in detail in Chapter 3. A bipolar. low impedance, silver/silver chloride wire stimulating electrode was placed under the median nerve proximal to the graft high in the axilla and a second bipolar recording electrode placed beneath the median nerve in the mid forearm (distal to the graft). Corresponding electrode positions were used for the con-

trol nerve. A ground electrode was inserted into pronator teres. Care was taken not to stretch the nerves and all tissues were kept moist with normal saline at 37°C.

Supramaximal constant current square wave stimuli of 50 μs duration at a frequency of 0.5 Hz were applied to the nerve and the resulting compound nerve action potentials (CNAP) were measured. CV_{max} was calculated in the usual way.

Nerve blood flow

This was measured using laser Doppler flowmetry with a Moor blood flow monitor MBF3D/42 (Moor Instruments Ltd. Devon. UK) simultaneously to both normal and repaired nerves just distal to the graft site. The method of use in peripheral nerves and the advantages and shortcomings of laser Doppler flowmetry have been discussed elsewhere (Drew et al. 1995). The laser probes were applied to the repaired nerves 2 cm distal to the graft and at an equivalent site on the control nerves. The mean reading over a 10-minute period for each median nerve was recorded. The Moor blood flow monitor provides a measurement of blood flow in 'Arbitrary Units' as opposed to absolute measurements of flow. For the purpose of this study, blood flow was expressed as a ratio of grafted NBF to control NBF. To reduce interference and 'light noise' the segment of nerve being tested was isolated from the underlying tissues with a short strip of aluminium foil. In all cases theatre lighting was directed away from the nerve as bright light significantly diminished flowmetry readings. For a fuller appreciation of the theory of laser Doppler flowmetry, see Drew et al. (Drew, Fullarton, Glasby, Mountain, & Murray 1995).

Morphometric studies

When physiological measurements were complete, specimens of nerve distal to the graft as well as the graft itself were taken. Nerve tissue from corresponding sites

of the control (left) median nerve was also harvested. Preparation, viewing and morphometric analysis of the specimens has been described in detail in Chapter 3.

Statistical analysis

This has been described in detail in Chapter 3.

In order to reduce 'within-groups' variation due to differences between individual sheep, each variable obtained from the repaired nerve was divided by its exact anatomical equivalent obtained from the normal, opposite side in the same animal. These fractional variables could thus be compared statistically with each other to determine the effects of the complicating injury.

RESULTS

Observation of each sheep on return for assessment showed no evidence of any functional deficit nor of the presence of any pressure sores or ulceration around the hoof or forelimb. At operation, all grafts were associated with varying degrees of scar tissue although there was no clearly discernible difference between the immediate and delayed repairs. The muscle grafts had remodelled with the appearance, colour and consistency of a thickened nerve. Nerve distal to the grafts tended to be slightly fatter with a more flaccid appearance than the control nerves at corresponding sites.

Figure 1 is a summary of the significant effects all expressed as fractions of the normal indices for each variable. A detailed analysis of the results and their specific significances may be found elsewhere (Fullarton, Glasby, & Lawson 1998; Glasby, Fullarton, & Lawson 1997; Glasby, Fullarton, & Lawson 1998; Lawson & Glasby 1995; Lawson & Glasby 1998). If one compares the graph of 'immediate' results with those in the 'delayed' group, it can be seen that for every

variable the values on the y-axis are depressed in the 'delayed' group. In addition there is a downward trend for all variables along the x-axis from left to right. These two features indicate that recovery is poorer as one progressed from the more simple to the more complex injury and that in every case, outcome is further worsened by delaying the repair of the nerve. Arterial injuries are associated with the worst prognosis as might be expected.

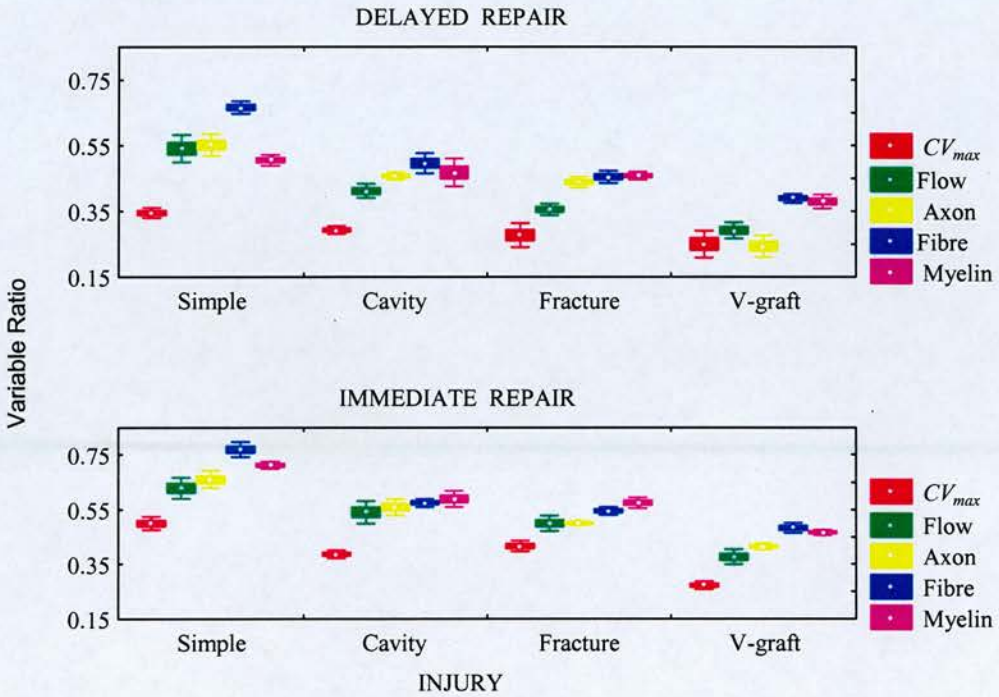


Figure 1

A summary graph of the significant effects differentiating the models for complicated injuries. In each case the mean (point) standard error of the mean (box) and standard deviation (whisker) is shown for the ration of the observed variable on the operated side divided by its anatomical equivalent on the normal side.

DISCUSSION

This series of experiments was designed to investigate the relationship between immediate and delayed nerve repair in association with a complicating injury. The result has been a clear demonstration of the inferior results obtained if the repair of the nerve is carried out at a later time than the primary procedure to deal with the associated injury. In addition, it has been possible to establish a clear hierarchy of the way in which the nature of the associated injury affects the outcome of repairing the nerve. The effects of immediate and delayed repair have been the subject of greater interest (Birch, Bonney, & Wynn-Parry 1998; Birch et al. 1986; Birch 1993; Brunelli & Brunelli 1990; Fullarton, Glasby, & Lawson 1998; Gattuso, Glasby, Gschmeissner, & Norris 1989; Glasby, Fullarton, & Lawson 1997; Glasby, Fullarton, & Lawson 1998; Lawson & Glasby 1995; Lundborg 1979; MacKinnon & Dellon 1988; Sunderland 1978; Sunderland 1991) but there is a surprising level of disagreement over clinical strategy. Kilvington (Kilvington 1905; Kilvington 1907; Kilvington 1908; Kilvington 1909; Kilvington 1912) considered both problems as part of his comprehensive and seminal, yet almost forgotten, study of peripheral nerve repair (Glasby & Hems 1993). He was quite clear about the disadvantages of delay in repairing nerves and advocated that delay should only be allowed in the most pressing and exceptional circumstances. Kilvington's study, though restricted by the available techniques and knowledge of the early years of the twentieth century, made predictions which have been almost all borne out with experience, modern technology and progress through research. He showed quite clearly that the results of repairing the nerve were always made worse by delay and by a complicating injury. Moreover, a combination of the two produced the worst results of all.

Kilvington's words were scarcely heeded and his work almost entirely faded into obscurity. Most of Kilvington's work was experimental and it is therefore perhaps not surprising that the dogma of the inter-war years should have derived from the First World War clinical experiences of Platt (Platt 1919; Platt 1921), who became a firm advocate of delayed repair (Glasby and Hems 1993). With hindsight it is easy to see that adherence to a strategy which was undoubtedly appropriate at the time nevertheless retarded progress as it became increasingly entrenched.

The difficulties of undertaking a controlled trial of complex nerve injuries, particularly in peacetime are enormous and the extent to which the results of the present study may prove useful in predicting clinical management depends upon the extent of any parallel between the factitious models of cavitation, fibrosis and haematoma, long bone fracture and vascular injury used here and the complicating injuries seen in clinical practice. That the latter are so diverse is the very reason why clinical trials are virtually impossible. Like all models of clinical situations. the present study may be open to the criticism that real injuries do not occur in precisely this manner, yet it is difficult to imagine and implement experimentally any other means by which at least a degree of scientific control can be exerted over a model for complex nerve injury.

The present study contains no information on how, if at all, the complicating injury should be managed. That is another study for experts in another field. This study has been involved with a 'worst instance' in order to quantify the effects of the complicating injury *per se*.

In the reality of clinical practice it is the degree and nature of the associated injury which usually determines the necessity for delay. There is little doubt about the sense of this approach when the associated injury is life-threatening or when the

local services (e.g. in the field of battle) are not appropriate for careful reconstructive surgery. It is likely that the detrimental effects of delay are directly related to the length of the delay during which time retraction of the stumps, intraneural fibrosis and neuroma formation is taking place. All of these processes act to impede both the first phase of nerve regeneration when pioneering axons are growing towards the distal Schwann cell tubes and target organs and the second phase of regeneration when maturation of nerve fibres, which have made substantive connections, is taking place. Modern anaesthesia and intensive care have greatly improved the safety of long operations and where at all possible, it seems that early repair will result in significantly better recovery and should be pursued with every effort. The use of biodegradeable tubes or wraps and tissue glue will reduce the technical demands placed upon surgeons repairing nerves and thus propitiate the earlier repair of nerves as a primary event, possibly even close to the field of battle (Gilchrist et al. 1998; Lenihan et al. 1998).

It is interesting to speculate upon how the complicating injuries and the delay in repairing the nerve contribute to the poorer outcome. Clearly, in the case of delayed repair, there is greater retraction of the stumps of the divided nerve and thus a requirement for a longer graft. Long grafts adversely affect the outcome of nerve repair (Hems & Glasby 1992; Hems & Glasby 1993a; Hems & Glasby 1993b) and indeed the quest for the long graft which functions well is the most pressing unsolved problem in peripheral nerve surgery. However, it is unlikely that this is the only factor operating in delayed repair and events taking place in the nerve itself and in the target organ are likely contributors. The worsening outcome with complexity of injury may reflect the reestablishment of nerve vascularity after repair. The progression of simple injury to cavity to fracture and to arterial injury may be expected to parallel more

extensive tissue damage, increasing scar tissue formation and poorer revascularization. The role of revascularization in peripheral nerve regeneration is poorly understood. It has been considered (Lundborg 1979) to be an important factor in determining outcome, but as yet methods of quantifying both vascularity and blood flow are inaccurate and the results are unsatisfactory. The finding (Lundborg 2000; Lundborg et al. 1997) that there was no difference in the results of repairing human median nerves by means of conventional epineurial microsuturing and by entubulation with impervious silicone rubber tubes is surprising if radially-entering local revascularization is to be implicated in affecting outcome. There is also no clear evidence, despite a large number of published studies, of any advantage in the use of either pedicled or free vascularized nerve grafts (Hems & Glasby 1993a). However, these studies were largely concerned with nerves repaired in near-ideal conditions and the absolute mass of tissue disruption was much less than in the experimental models considered here. The question must be raised as to whether the effect of the complicating injury is mediated simply by the extent of the *damage* to tissues around the site of nerve repair or whether the *healing* process associated with the complicating injury somehow diverts the process of revascularization away from the nerve to produce a poorer result. If this is the case, the vascular injury must be regarded as a special case, for in this instance there is no means of *supplying* vascularization because of the loss of a local large feeding vessel. In the present study there was no obvious difference in the amount of intraneural connective tissue in the various samples, though this was not formally quantified. A difference might have been expected if there were differences in vascularity between the groups. One of the problems associated with laser Doppler flowmetry is the restricted penetration of the laser light into the tissue, so that surface and superficial vessels contribute more than

deep vessels to the final computation of flux. It is interesting, therefore, that in the present study a gradient of flux was seen from the more simple to the more complex injuries, as it is the superficial vessels which one might expect to be affected most by events in the surrounding tissues. In the case of the arterial injury, where the local vascularity was most dramatically disrupted, the poorest recovery was seen. This finding supports the implication of a vascular factor in the outcome, but the exact mechanism remains to be elucidated.

The experiments described here were an attempt, using a large animal model, to test the hypothesis that the presence and the nature of a local injury which complicates a nerve injury, and a consequential delay in the repair of the nerve, separately and additively contribute to a worse outcome. The conclusion from the experimental results is that the hypothesis is correct and that since primary nerve repair offers the greatest advantage to recovery, it should be carried out wherever possible.

CHAPTER 9 — EVALUATION & CONCLUSIONS

Ne supra crepidam sutor iudicaret.

(Pliny The Elder, A.D. 23–79, *Historia Naturalis 85*)

PLINY reminds us that we should not operate beyond our resources and experience. This is essential advice in any consideration of experimental data. Chapters 4,5,6,7 and 8 have been concerned with the application of the tests described in Chapter 3 to the models defined in Chapter 2. It is now appropriate to discuss the usefulness of the various tests in the light of what they revealed about the specific injuries and the methods of their repair.

EVALUATION OF THE SHEEP MODEL

One of the main objectives of the present study was to assess the sheep as a model for the surgical repair of nerve injuries. Much was said in Chapter 2 about the sheep model in general and this need not be repeated here. It was known at the outset that the sheep provides a more realistic surgical subject if the final objective is to be human use. Most of the ovine nerves considered above were of approximately the same size as their human counterparts and the time-course for recovery of motor function was not markedly different from that found in humans.

A few differences exist in the details of specific anatomy. For example, the sheep brachial plexus begins at C6 rather than C5. This does not, however, make it equivalent to the 'post-fixed' plexus occasionally seen in humans and it may not be considered a model for that anomaly.

The overwhelming problem with the sheep model is that it does not allow the sensory system to be considered adequately. Of course this is true of most animal models and so is not a specific disadvantage. However, even for non-subjective studies on sensory

nerves such as the measurement of purely sensory conduction velocities the sheep is at a disadvantage because the sensory supply to its hooves is so rudimentary. Below the *Primates* there is really no adequate model for sensory studies and the task of using primates for animal studies in the U.K. at present is virtually beyond contemplation. There is, of course, the option of using humans for this kind of study though, as in all nerve surgery, the problem of the heterogeneity of injury means that controlled groups cannot easily be established. Nevertheless such work has led to significant progress (Tubiana, Thomine, & Mackin 1996). Unfortunately clinical timetables and finances do not lend themselves to this kind of work which has been unfairly neglected as a consequence. This is almost certainly a false economy as more diligent studies of this nature would surely lead to a better understanding of the natural history of nerve injury and repair.

Comparison with small animal models

Again, discussion on this subject has been undertaken in Chapter 2. One important fact has emerged from the present sudy however and this is that it must not be thought that the use of large animals allows smaller group sizes to be used for evaluating the models. With the benefit of computerized Power Studies it is now possible to make very clear predictions, from past experiments and from pilot-studies of the group sizes which are needed to give useful staistical results. This must be viewed in the light of the particular tests used and the questions which they are employed to answer. Over the past fifty years many experimental series using just six rats have entered the lierature. At EPNRL we have used such experimental protocols to good effect in many cases but too often the final results have been on the borderline of statistical significance, raising new questions rather than answering old ones. This has sometimes led to the undoubted false economy of the work's having to be repeated.

The move to the ovine model was first undertaken because the anatomy and time-course of recovery were more akin to the human. However it would be wrong to deny an early hope that the more stable nature of the sheep model and the ease with which sheep could be looked after and assessed, engendered a hope that one could use small groups more satisfactorily than hitherto with rats and rabbits. This was not the case. While there were fewer problems resulting form death, disease, fighting etc. the statistical tests, and later on Power Studies showed up the advantage of a large group in ensuring clear results. The final decision, vindicated by the present work, was that a group size of 12 animals allowed for a good statistical comparison of all of the variables of interest along with the possibility of loss of up to two animals through whatever misfortunes might occur.

It is possible, therefore, to make the categorical recommendation to future workers that the sheep model, as used for the kind of experiment described here, in groups of twelve or more animals, may be relied upon to disclose significant changes in the values of the measured variables which are adequate to test the models under consideration.

The sad irony of such a remark must not go unmentioned. At the time of writing EPNRL is about to close. The University of Edinburgh feels obliged to identify certain avenues of research which it would wish to support in preference to others and without regard for the past merit of individual workers or groups. This obligation is undoubtedly the result of financial pressure rather than scientific rigour exemplified by the fact that research leading to collaboration with external businesses, the setting up of biotechnology companies and the establishment of patents — i.e. those ventures bringing money into the Universities — is now invariably identified as having the greatest 'scientific merit'. 'Bibliometrics' has been supplanted by plutolatry as the

Holy Cow of university research. Research into the clinically useful but numerically small field of peripheral nerve injury has been regarded as not meeting these criteria and is thus to fall by the wayside. Regrettably, the universities have applied such floccinaucinihilipilification liberally and nerve repair is by no means the only subject to suffer this fate. As the work of EPNRL has taken up a substantial proportion of the large animal experimental resources within the University, it cannot now be long before that resource is closed 'owing to lack of funds' and thus denied to future workers. The tragedy is compounded by the fact that in the U.K. there are now few large animal experimental facilities and these are more often than not embattled by animal 'rights' terrorists or battling against inflating university costs: particularly in the cities of the south. Edinburgh was, for the period 1987–2004, at least, free of both these ills.

Thus, it seems likely in the U.K, that the laboratory rat, despite all its now well-documented shortcomings, will remain the animal of choice for studies of peripheral nerve regeneration — *sic transit gloria mundi*. It remains to be seen whether the *caveats* discovered here by the use of a better model, no longer accessible, will be heeded.

EVALUATION OF MODELS OF INJURY & REPAIR

With the exception of those for neurapraxia and the Sunderland IV lesion these are not new. However the material used for the entubulation experiments, controlled release glass (CRG) is new. Detailed evaluation of the various models is discussed in Chapters 3 and 4.

Models of injury

Neurapraxia.

Hitherto there has been no convincing model for neurapraxia. This is partly owing to the varied and transient nature of the condition and partly to the difficulty in applying an insult which is time-limited in its effect. The large animal model greatly facilitates the application of the CRG ligature and allows its tightening to be coördinated with neurophysiological studies to indicate that there has been a localized slowing of the impulse. There is still some controversy as to the exact nature of the histological injury in neurapraxia though myelin fragmentation is its cardinal feature. It is not possible from the studies reported here to assert that this model is histologically identical to clinical neurapraxia: the model is, however, identical to the clinical condition in respect of its electrphysiological properties and in its time-course. As such, it is a valuable addition to the researcher's armamentarium.

Axonotmesis

Traditionally the crush-injury has been employed experimentally and given a variety of names from axonotmesis to the invariably unspecified 'axotomy' which, as it is frequently passed off as a model for axonotmesis, must be anything but that. If the literature is perused for only a short time it becomes apparent that the nature of the instrument used and the timing and pressure used in the insult vary greatly indeed. Although it was not attempted here it may be possible to apply a tighter CRG ligature made with a longer solution-time to mimic, at least, the electrophysiological properties of axonotmesis and this may present a more reproducible injury than that seen in previous experiments.

Sunderland IV lesion

Some of the reported models for axonotmesis mentioned in the preceeding paragraph may have been, perhaps more often than not, Sunderland Type IV injuries where only the epineurium was in continuity. In Chapter 7 where a model for the traction injury of the brachial plexus was sought it was possible, at least to a degree, to control the pressure applied to crush the nerve and the time for which pressure was applied. Histological confirmation of a Sunderland type IV injury, unlike neurapraxia, is a simple task. This is thus seen to be a very reliable and accurate model for this serious injury. Again its production in a large animal is more dependable than in the rat.

Neurotmesis

A model for neurotmesis is always simple to create though less simple to repair. The principal concern with this injury, where experimental reproducibility is needed, is in obtaining a uniform degree of contraction of the stumps. Unfortunately this can never be guaranteed. Additionally the degree of overlap of the stumps by loose epineurium may also be inconsistent. Both of these factors can affect the neurroraphy or fascicular repair which one might undertake. In the present experiments the use of the Meyer Neurotome, obtainable as a sterilizable set with ring diameters of 1.0mm to 5.0mm, resulted in near reproducibility at least in the appearance of the stumps. This greatly facilitated epineurial repair.

Models for repair

Suture

So much has been written on the technique of nerve repair by suture and so much remains uncertain: the dilemma over epineurial *versus* fascicular repair continues to

grind on relentlessly. The debate is perhaps more often about personalities than about surgical technique. The *Vox Dei* is badly needed for the forthcoming generations of surgeons who will have graduated from 'core courses' rather than have learnt by experience. It is interesting to note that of the two most experienced peripheral nerve surgeons known to the present author one, in London, almost never uses a microscope relying for assisted vision on loupes; while the other, in Vienna, uses the microscope for even the largest nerve. Their results are equally good and better than those of most of their peers. This confuses the trainee who needs all the assistance he can find but wishes also to emulate his masters. In the laboratory, much can be done to perfect techniques and to make comparisons under controlled conditions. From experience of such formalized studies it is a simple matter to recommend the rigorous employment of strict microsurgical techniques with microscope-assisted vision and a sticking to the technique of epineurial suture except and only when a very high degree of skill has been obtained and the case has been properly made for fascicular repair. In most hands an attempt at fascicular repair does more harm than good. However it is easy to make such pronouncements and a different matter to see them realized in clinical practice.

In the present experiments successful repair has been identified with epineurial suture and found to be reproducible. It has thus formed the 'conventional repair' in many experiments, with which more novel techniques have been compared. It is with this model in mind that the diagnostic and follow-up tests must be considered as this method will, for a long time still to come, remain the standard against which all techniques for nerve repair are judged.

Grafting

The same *caveats* apply (twice in each repair) but failure to observe them invariably results in more than twice the morbidity. In comparison with simple neurroraphy grafted nerves do badly with the length of the graft being roughly inversely proportional to the success of the outcome. In the present experiments there was a consistent finding that where grafts were compared with any technique embodying a single suture line, the outcome was poorer. Whatever combination of methods of injury, repair and assessment were tried, this was the invariable result.

Entubulation

Having been an early method of nerve repair, entubulation fell from favour with the rise of microsurgery but is now experiencing a renewal of interest. Much credit for this must go to Lundborg's claim for the virtue of a small gap in concentrating the chemical factors necessary for optimal regeneration although there is latterly much scepticism about this (Lundborg et al. 1982b; Lundborg et al. 1982a; Lundborg et al. 1997; Lundborg 2000; Lundborg, Dahlin, & Danielsen 1991; Lundborg & Hansson 1980). Based on this idea, the use of silicone tubes was translated from the laboratory to clinical practice and turned out to be a disastrous mistake as many of the patients required a second operation for the acute nerve compression caused by the retained tube. Nevertheless this work was seminal in stimulating a useful flurry of work upon biodegradeable tubes which has been altogether more promising. The problem with biodegradeable tubes is, however, that they are seldom impervious/impermeable and the retention of chemical factors within a 'nerve-friendly' environment was the foundation of Lundborg's theory. Leaky tubes do not seem to have made much difference to clincal outcome though as might be expected fierce competition between

different researchers (and their manufacturing 'backers') may have led to precipitous conclusions in a number of instances. The interest in 'growth factors' predicted as the salvation of nerve injuries about 12 years ago has dwindled seriously of late as the benefits have not been shown to occur or, at least, where they have, they have been so negligible as not to justify the effort and expense of their use[21]. The idea of entubulation has moved to the simpler and, for the time being, more practical view that it may serve as a mechanical means of repairing nerves on the battlefield or in the 'third world' or indeed in an impoverished National Health Service. The CRG entubulation reported in Chapter 3 goes a long way to achieving this simple but valuable goal. All that was necessary after proving that the CRG was appropriately biodegradeable and non-toxic, was to show an outcome at least as good as that seen with conventional neurorraphy. This has been achieved and the CRG wrap[22] has proved an excellent choice for entubulation. Recent work at EPNRL has shown that the wrap and nerve may be securely and effectively attached with currently available tissue glues such as 'Tisseel'.

EVALUATION OF THE TESTS

Of the considerable battery of tests which, on the basis of past work, were investigated here, only a small number showed themselves to be of use.

[21] This was predicted by the present author at that time, when he chose not to go down the 'growth factor path' and was greatly criticized for it, not least by University Management who doubtless saw a pathway to fortune. The view at the time, as realized now, was that '...increased growth could equally mean increased cellular chaos and a useful growth factor would be one that controlled specificity of growth rather than merely stimulating it in the generality...'. This was soon shown to be the case.

[22] A wrap is infinitely easier to use than a tube and also cheaper to supply.

Tests for laboratory use

Isometric tension tests proved virtually useless in the experiments performed here. That is not to say that they may not be valuable in assessing fundamental properties of muscle reinnervation and in assessing how much work a muscle can do after its nerve has been repaired. There are many instances in work published from EPNRL where these tests have been of value. In the present context of comparing methods of repair they had nothing to offer.

The morphometric study of nerves after repair and regeneration remain the 'gold-standard' of the **anatomy** of recovery but of nothing else. Findings here have proved helpful in backing up the measurement of physiological variables where true function is being assessed. It is important to correlate those variables which are measurable in the clinic with anatomical changes for if these fall into line, one can expect regeneration to be taking is proper course. It may be possible, in the future, for some similar properties to be measured non-invasively by perhaps MRI and this would be a source of confirmation of findings made by electrophysiological means.

Tests for clinical use

In the present experiments only CV_{max} emerged as a clearly useful discriminator for all the different methods of repair. Many of the tests were able to discriminate between normal and injured/repaired nerves but this would be simply stating the obvious.

CV_{max}

CV_{max} is the most logical measurement that can be made on the nerve itself rather than on the target organ, to test the ability of a nerve to conduct. It is therefore intellectually pleasing to record that in the present work this test emerged every time

as the most discriminatory variable which could be measured. This finding is made the 'winner'. This was made all the more attractive by the fact that CV_{max} is easy, cheap and non-invasive to record. It is a tragedy that more use is not made of CV_{max} in clinical practice though it is true that in many centres it is virtually impossible to coördinate surgical and neurophysiological Outpatient clinics. However today there are many small hand-held electronic instruments which allow the simple measurement of velocity or latency and which it is scacely beyond the ability of most surgeons to use. Recording this variable before and after treatment routinely would be of enormous benefit.

CV_{Dist}

CV_{Dist} is potentially a good test of peripheral nerve function. In nerve injury, however, the injury was too severe for the test to be of more use than the measurement of CV_{max} although it was a good discriminator for neurapraxia and axonotmesis. CV_{Dist} may be of value in the assessment of neuropathies, poisonings and such conditions. It is relatively non-invasive. Further investigation of this means of assessment is awaited.

TSJ/VAJ

Previous work had suggested that TSJ would be a most useful discriminator of the time-course of neuromuscular regeneration (Lenihan et al. 1997; Lenihan, Sojitra, & Glasby 1998). However this was not borne out by the present experiments other than those relating to the brachial plexus. There is an explanation for this: TSJ is a useful means of assessing neuromuscular stability whilst the regeneration process is taking place but loses its discriminatory power once the process is complete. In the case of the median and facial nerve experiments it is likely that regeneration had reached its end-point in all of the cases studied. Indeed this was the aim of the experiment and

reason for choosing the particular regeneration period. In the brachial plexus experiments, although regeneration was far enough progressed for distal muscles to be activated, it is likely that the process had not reached completion. TSJ has been therefore somewhat underestimated as a test by the present work. Its value (as VAJ in the clinic) would undoubtedly be established in the follow-up of slowly regenerating nerves i.e. in proximal lesions.

EVALUATION OF THE SPECIFIC EXPERIMENTAL MODELS

These experiments were set up to study the 'natural history' of nerve injury and repair using a large animal model. The aim was to mimic as nearly as possible the *Sitz im Leben* in which these injuries are encountered in clinical practice without the attendant heterogeneity which, outside the laboratory, is inevitable.

The results allow us to know for certain, after much past dubiety, that these nerves never, even with a range of treatments and after testing by a variety of means, return to normal. Of course, this is not a new fact but it has not before been determined with this level of formality. A second finding is that there is really not a great deal of difference between the various methods of repairing nerves. Good — by which is meant technically competent— suturing or good entubulation both do equally well; the results are far from ideal but are the best that can be achieved.

Immediate and delayed repair

The present experiments make an overwhelming case for immediate repair wherever this is possible. In many critical situations this may not be possible. All the more important, therefore, is the finding that there is nothing to choose between repair by suture and entubulation for the latter may make possible early repair in at least some of the situations where it was hitherto unthinkable.

The brachial plexus

This is, above all a proximal injury and as such suffers by the longer distance over which regeneration must occur. The mechanism of injury — avulsion or Sunderland Type IV — is also a factor in the appalling prognosis.

Accurate, early diagnosis is essential and is often difficult because of the nature of the injury. MRI scanning is invaluable in allowing avulsions to be seen. F wave measurement is also potentially useful but can be notoriously difficult to obtain. CV_{max} cannot usually be measured because of the problem of electrode placement though the technique in the modified form of evoked potential measurement may have much to offer. In the present experiments TSJ emerged as a useful tool specifically in the follow-up of these injuries and it simpler and less painful relation, voluntary jitter may have much to offer in the assessment of outcome during the regenerating phase after repair. The rôle of evoked potentials should be investigated.

Complicated injuries

In the present experiments a formal comparison of the simple injury to a nerve was made with cavitation, fibrosis and haematoma, with associated long-bone fracture and with arterial injury. A worsening prognosis was seen in parallel with this list of injuries. Moreover early repair was always associated with better outcome than late repair. It was regrettable that infection could not also be considered.

The comments made above in relation to immediate and delayed repair apply here with even greaer force. Again entubulation may offer some means of easing the situation.

UNANSWERED QUESTIONS

The work presented here contains no information on what must still be regarded as the two greatest outstanding problems in peripheral nerve repair.

The long gap & 'enhancement'

Technical advances relating to this problem reached a plateau some years ago. Many different forms of graft have been tried and, since the reawakening of interest in entubulation, many permutations of tube and tube-contents. None has been associated with significant improvement and outcome remains overwhelmingly poor.

It seems, instinctively, that this problem may await the advances in stem cell manipulation. Attempts at enhancement with growth factors have been disappointing. However as with the latter the problem for stem cells may be that the technique, while inducing nerve growth aplenty *per se* and perhaps also better maturation of pioneering axons, does not induce a level of specificity which is commensurate with the degree of sophistication needed for a functioning peripheral nerve.

THE FUTURE

This cannot, of course be predicted, but certain points can be identified as potential areas for research and progress. However we should not be scientifically naïve and suppose that other factors will not have their effect. Academic freedom has been wiped out from British universities by the noxious advance of the Research Assessment Exercise and 'themed' research driven by unscholarly, financial forces has replaced it. If one supposes that, as in the past, grains of important and useful research emerge, in an almost Darwinian evolutionary way, from a greater, freely-operating mass of scientific endeavour (of which the outcome could not possibly have

been known at the outset), then one must conclude that the stifling of such a 'freemarket' — to use a cliché much in vogue — will result in fewer discoveries. It is not clear that 'theming' research before it is carried out will necessarily push to the fore the most valuable and relevant discoveries.

Scientific factors

Undoubtedly molecular biology is yet to make its mark on nerve repair. At present the peripheral nervous system has not attracted these scientists as they pursue richer rewards in the brain and spinal cord. This is a pity as there is much on offer. The poor results of growth factor experiments does not appear to have dampened their ardour. A more realistic approach appears to be emerging as the limitations of the 'new biology' come to light.

Distasteful assertions about the potential achievements of new experimental methods have sometimes been made in an attempt to obtain funding. This is, outrageously, becoming more common. Panels of 'experts' should be a means of avoiding this. Nevertheless this is not the case and the present author well remembers hearing, some eleven years ago, the prediction that 'paraplegia would be curable within ten years' being made (by one who should have known better) at an international conference. The disappointment that statements like this bring upon patients makes such arrogance shocking and inexcusable.

With the exciting advances currently being made in stem cell culture and manipulation there may at last be a route for improving peripheral nerve regeneration. However, the transition from *in vitro* to *in vivo* and even then to the human, as so often before, may prove a difficulty or even a dead-end. It is to be hoped that this advancement will be spared from the rashness which has so fruitlessly befallen other

potentially useful and exciting avenues of research. In experimentation, patience undoubtedly benefits patients.

Political/fiscal factors

There is a distasteful element in scientific research in the United Kingdom today. Government policy in what is erroneously called a 'freemarket economy' has resulted in severe underfunding of the universities and the shifting of the provision of resources for research to the researcher. Little or nothing is provided '*gratis*' by the employing institution. The word 'fund', once a noun, has become an over-used verb. As a result 'fund-raising' for a particular research project has become devolved upon the researcher rather than the institution. In theory, in a truly free-market economy, there should be no problem with this. It is financial/scientific Darwinism and there is nothing wrong with that. However in the reality of life other forces become active. The most powerful, and the most offensive of these are fashion and 'media-pressure'. Science has, in the lastter half of the twentieth century been surprisingly affected by these. This has not necessarily been a bad thing. In the biomedical sphere, the 1950s saw a massive development in the biophysics of nerve conduction with exciting and fundamentally important work from e.g. Hodgkin and Huxley and the first Lord Adrian. This continued through the 1960s and early 1970s with e.g. the important work of Katz and was developed to encompass the brain and spinal cord. Our knowledge of the central nervous system developed considerably. By the early 1980s, neuroendocrinology had become *primus inter pares* and then neurobiology[23] became the centre of attention. By the 1990s, molecular biology had emerged, and this undoubtedly important development has, since that time, expanded to such a degree

[23] Never adequately defined to this author's satisfaction.

that it has become the centre of gravity of biological science. It would be invidious to suppose that much of this honour is not deserved, but enthusiasm has now become so great that the jettisoning of other branches of (neuro)science has occurred, almost unnoticed. There can be no question but that molecular biology will in time[24] offer the solution to many unanswered questions about nerve regeneration; it would be utterly naïve to suppose that it is the only avenue of research worthy of support. Yet this is what appears to be happening and one cannot help but make the cynical connection between such a development and the fiscal usefulness which underlies it. Molecular biology is cheap to run, the universities are producing many graduates equipped for a career in that field who have not needed the expensive background of a medical training. Most experiments take place in bottles and animals are not needed: there is, therefore, no sensitive political element to be considered: one can shut one's mind to all else. On the credit side many molecular biologists have chosen to associate their work with the founding of 'biotechnology companies' and so money can be made and directed to the universities which supported the initial venture. In the present climate of university poverty it is of no surprise that such a situation is overwhelmingly attractive and has thus been encouraged to develop at the expense of more conventional methods of research. But at what real cost? Whether such narrow support will, in the future, turn out to be prudence or folly remains to be seen. In the meantime the profligate closure of more conventional research groups and animal facilities remains a souce of concern.

[24] And there lies the question, how long? Many grants have been acquired on the promise of a 'clinical' breakthrough (cf. cystic fibrosis) when, in fact, there are still many and great fundamental and clinical problems to be overcome.

GENERAL CONCLUSIONS

It is meet, here, to reflect upon achievements, over the last twenty years, both in the generality and in respect of the work presented in this thesis. Without question the most important advances in peripheral nerve surgery during the twentieth century were the product of an immense impetus generated by the tragedies of the two world wars. So often, progress in medicine has been the result of conflict. Two factors emerge above the others. They are, better diagnosis and the application of technological advances to the practice of surgery: both were forseen by Kilvington though he was, for lack of modern resources, unable to develop them himself. Where recent research has been less successful is in documenting the 'natural history' of nerve injury and its repair and it is specifically to this problem that attention was turned in the EPNRL and in the work presented in this thesis. The philosophy behind this has been that, because there is such an heterogeneity[25] in nerve injury and in its treatment, laboratory studies may offer a means of 'standardizing' or at least unifying a clinical approach such that certain predictions can be made and transferred with benefit to the clinical arena.

In one sense the outcome of the last twenty years' work in EPNRL has been a disappointment. If one were to read this thesis in isolation one might conclude that the single positive outcome is the undoubted assertion that the study of CV_{max} is, alone, the most useful test that one can peform both to establish the nature of the injury and the progress of the treatment. This is a good thing because CV_{max} is the easiest test to carry out: it is therefore all the more depressing that so few surgeons in the U.K. make adequate use of their neurophysiological colleagues and even more depressing to find that this is not the case in many other countries. One can feel

[25] Sometimes unavoidably real and sometimes generated by unthinking practice.

secure in asserting that if nothing else is determined by this thesis, the place of the measurement of CV_{max} is so firmly established that its omission in management must be viewed as gross inadequacy. A secondary finding here is that in certain cases, the measurement of jitter may be regarded as a useful adjunct to CV_{max} because it is particularly sensitive to the progress rather than to the end-point of the regenerative process. This is especially true in proximal lesions. Its use may therefore be a helpful indicator as to how treatment is progressing and it may indicate the need for a change in strategy before all is lost to the inexorable progression of degeneration and fibrosis.

The second way in which this thesis may be regarded as helpful, despite a large number of negative findings, is that it encompasses, in a wholly controlled and scientific way and in a relevant model, a large number of questions which needed to be answered. These relate both to the natural history of nerve injuries and their repair and to what tests may or may not be useful in their follow-up. We may now,with a clear scientific conscience, discount a number of avenues of research and potential management which had once been thought to be helpful: in particular those relating to isometric tension measurement. Likewise the morphometric studies have, over time, served their purpose in showing where differences exist but are of no use in the clinical setting. We must guard against proliferating their use simply because they are easy to measure in small research projects and have a long pedigree. There remain CV_{max} and TSJ/VAJ (and perhaps CV_{Dist} in very specific cases) as significant and useful comparators of diagnosis and treatment. It is crucial that more extensive use should be made of these important and valuable tests.

But what is left? The reality of all this is that we are almost no further on in answering the question 'why is there such a discrepancy between the form and function of normal nerves and of repaired/regenerated nerves?'. The answer is almost certainly

that we can never hope, using human art, to reproduce the sophistication of ontogeny: or perhaps we cannot yet devise the right experiments to elucidate these phenomena. A greater scientist than the present author was well aware of this and it is fitting to remember his words — which he wrote in his conclusion to one of the most important scientific texts ever:

> ...et ſenſatio omnis excitatur, et membra animalium ad voluntatem moventur, vibrationibus ſcilicet hujus ſpiritus per ſolida nervorum capillamenta ab externis ſenſum organis ad cerebrum et a cerebro in muſculos propagatis. ſed hæc paucis exponi non poſſunt; neque adeſt ſufficiens copia experimentorum, quibus leges actionum hujus ſpiritus accurate determinari et monſtrari debent.

(Isaac Newton, *Philosophiae Naturalis Principia Mathematica* 1726; — concluding remarks[26]).

[26] ...and all sensation is excited, and the limbs of animals move at the command of the will, namely, by the vibrations of this spirit being propagated through the solid fibres of the nerves from the external organs of the senses to the brain and from the brain into the muscles. But these things cannot be explained in a few words; furthermore, there is not a sufficient number of experiments to determine and demonstrate accurately the laws governing the actions of this spirit.

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APPENDIX 1

GENESIS XXXII : 32

> 'Therefore the children of Israel eat not of the sinew which shrank, which is upon the hollow of the thigh, unto this day: because he touched the hollow of Jacob's thigh in the sinew that shrank.'
>
> *Authorized Version*

The interpretation of this story in the Bible is something of an exegetical conundrum as much in respect of the theology as the language. Jacob, on his wanderings, is confronted by an angel with whom he wrestles throughout the night. Christian art often portrays the archangel Chamael (or Chemuel) in this rôle though other interpreters have claimed it was God Himself. The angel gave to Jacob the new name of 'Israel' and the significance of the passage appears to be the assertion of man's resistance to God and his absolute freedom to accept or reject God. Occasionally this story is seen more simply as the struggle between Vice and Virtue (Taylor 2003).

It seems that, as the wrestler could not prevail over Jacob, he resorted to 'below the belt' tactics and struck Jacob in the groin. It is interesting to speculate upon the injury that resulted. There are several contenders depending upon whether one believes that Jacob sustained a musculo-skeletal injury or a nerve injury. If one examines the ancient texts and **more** recent renderings, the odds appear to be just about even.

In theory, the Hebrew text of the Torah should be the most authoritative source, but it must be remembered that there are no manuscripts available which are contemporaneous with the historical events recorded in the Old Testament. The Masoretic Texts from which all modern versions of the Hebrew Old Testament are derived were the result of extensive recension and emendation carried out by the Masoretes much later, between the 6th and 10th centuries A.D. (Peake 2001). The oldest extant texts of the Hebrew scriptures are those of the Essenes discovered at

Qumran which are known as the Dead Sea Scrolls. These were written sometime between the second century B.C. and the end of the first century A.D. (Vermes 2000). The following is a literal translation of Genesis XXXII : 32.

. The words נָשֶׁה (tendon) גִּיד (sinew) and יָרֵךְ (hip or thigh) are quite clear in their meaning (Bible 1987; Brown, Driver, & Briggs 2001; Strong 2003; Wigram 1874)[2].

כֵּן לֹא־ יֹאכְלוּ בְנֵי־יִשְׂרָאֵל אֶת־ גִּיד הַנָּשֶׁה אֲשֶׁר עַל־

on that/which the tendon sinew-of [ACC] Israel sons-of they eat not this for

5384

כַּף הַיָּרֵךְ עַד הַיּוֹם הַזֶּה כִּי נָגַע בְּכַף יֶרֶךְ יַעֲקֹב בְּגִיד

on Jacob hip-of on he for the this the day to the hip hollow

sinew-of socket-of touched / thigh -of

3409 3709

הַנָּשֶׁה

the tendon/vein

There is thus no suggestion from the Hebrew text that the 'sinew which shrank' was specifically a nerve. If one presumes the 'hollow of the thigh' to be the groin and taking into account the medical history, the tendon of adductor longus seems a strong candidate though it is hard to imagine that the equivalent tendons from sheep and cows would be cooked and eaten as they might be expected to be somewhat indigestible. Some authors have suggested that the injury may have been a dislocation of the hip but this injury requires a very considerable degree of force to be applied and is an unusual consequence of wrestling. It seems likely that the problem may have been a 'groin strain': the injury beloved of football players. Further support for this view is evinced from a study of the Revised Standard Version (1884) where the phrase 'sinew of the hip' appears twice in the above passage.

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[2] Hebrew, it should be remembered, is read from right to left.

This is all very well until one consults the Septuagint (LXX). Although the LXX is written in the κοινη Greek of the Hellenistic period and thus of Ptolomaic Egypt, the extant early texts are considerably older than the Masoretic Hebrew texts (Septuagint 1851).

Ἐνεκεν τούτου οὐ μή φάγωσιν υἱοὶ Ἰσραὴλ τὸ νεῦρον, ὅ
therefore of this not they eat sons [of] Israel the sinew which
ἐνάρκησεν, ὅ ἐστιν ἐπὶ τοῦ πλάτους τοῦ μηροῦ, ἕως τῆς
was benumbed which is on the flat [part] of the thigh until of the
ἡμέρας ταύτης, ὅτι ἥψατο τοῦ πλάτους τοῦ μηροῦ Ιακὼβ
day this because he grasped the flat [part] of the thigh Jacob
τοῦ νεύρου, ὅ ἐνάρκησεν.
of the sinew which was benumbed

The sense is quite different here because the word ἐνάρκησεν, the imperfect tense of the verb νάρκειν to 'benumb' is, of course, the origin of the modern word 'narcotic' and has no connotation of 'straining' or 'dislocating'. It strongly implies that the injury had caused some sort of change in sensation and this would need nerves in the modern sense. The Greek word νεῦρον is usually translated as 'sinew' in classical literature (Liddell & Scott 1968) and this meaning would have been expected to have persisted beyond the time of writing of the LXX (Danker 2000; Hatch & Redpath 1897; Septuagint 1851). It is interesting to speculate, therefore about how the choice of ἐνάρκησεν came about.

The Vulgate (St Jerome, A.D.341 – 420) offers further confusion (Vulgate 1994). The word 'nervum' was used in Latin in much the same way as νεῦρον in Greek, most often to mean a 'sinew' but here in verse 32:

Quam ob causam non comedunt filii Israhel nervum qui emarcuit in femore Iacob usque in praesentam diem eo quod tetigit nervum femoris eius et obstipuerit.

Vulgate

The Douay Rheims Bible renders this as:

> Therefore the children of Israel, unto this day, eat not the sinew, that shrank in Jacob's thigh: because he touched the sinew of his thigh and it shrank.

Two conflicting ideas emerge in the Latin. In the first part of the verse the word emarcresco (wither/dwindle away) is used to suggest the 'sinew which shrank' in the Hebrew sense, but at the end of the verse it is referred to again by the word obstipesco (to become senseless/be benumbed/be stupefied) suggesting a loss of sensation. It is tempting to think that, in fact, these two words are suggesting a mixed femoral nerve lesion with loss of sensation and the wasting typical of a lower motoneurone injury. However such wasting would not be apparent immediately and would have taken some weeks to develop. Ochs, (Ochs 2004), tends to the view that the injury involved the sciatic nerve despite the reference in the Vulgate to 'nervum femoris'. To justify this he cites the Jewish butchers' custom of 'porging[3]' which is the removal of the sciatic nerve from the rump thus allowing the latter to be consumed under Kosher Law. However it seems unlikely that anyone would consider the buttock, a well-rounded structure if ever there was one, to be described as the 'hollow of the thigh. The groin seems a much more logical contender.

Luther sits on the fence, (though perhaps not on the 'hollow of his thigh), while the French Jerusalem Bible quite clearly believes the sciatic nerve to have been involved.

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[3] Stripping out the sciatic nerve.

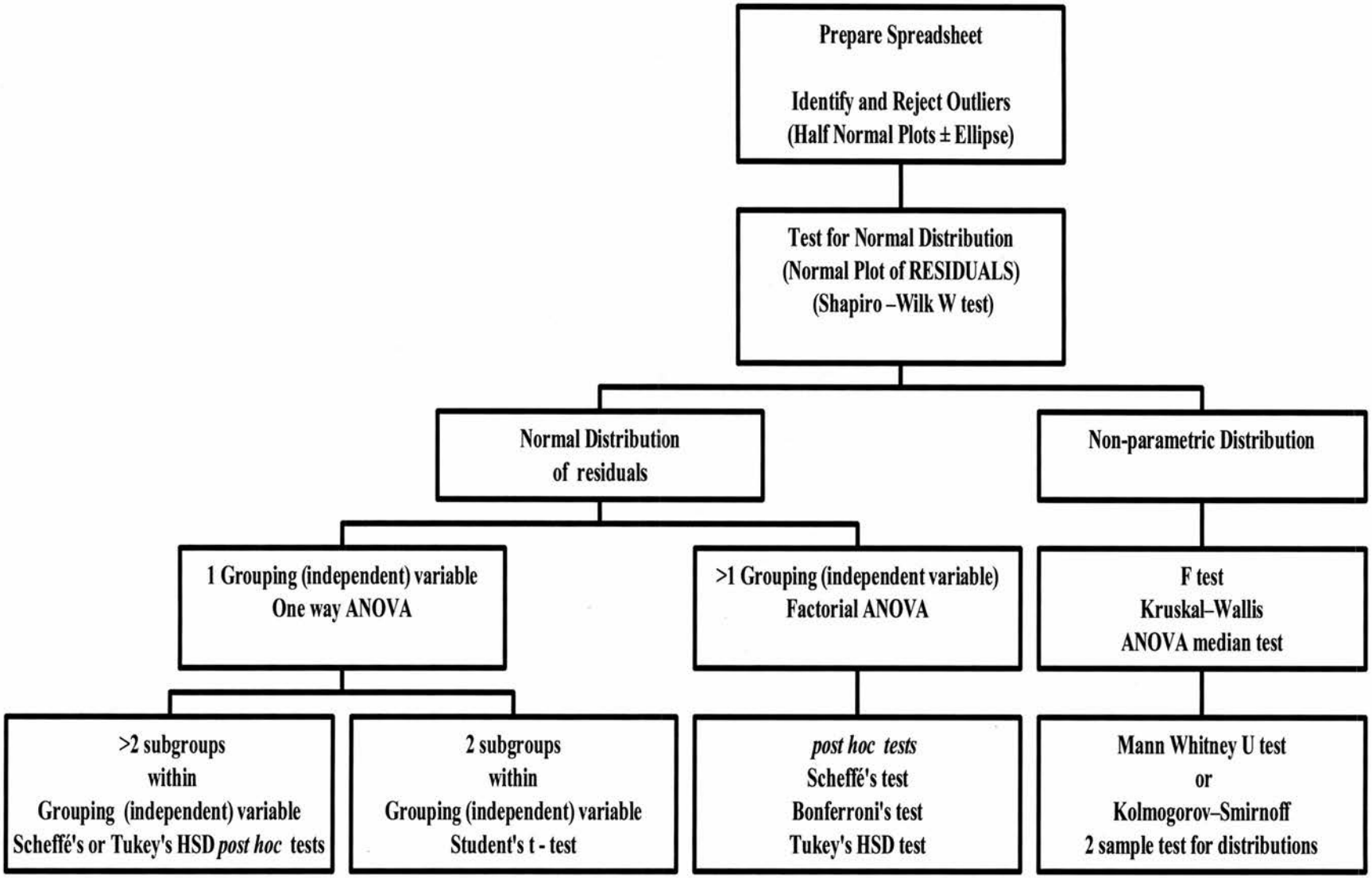
Daher essen die Kinder Israels keine Spannader auf dem Gelenke der Hüfte, bis auf den heutigen Tag, darum dass die Spannader an dem Gelenk der Hüfte Jakobs ward gerühtet.

Luther's Bible

C'est pourquoi les Israélites ne mangent pas, jusqu'à ce jour, le nerf sciatique qui est à l'emboîture de la hanche, parce qu'il avait frappe Jacob à l'emboîture de la hanche, au nerf sciatique.

Jerusalem Bible, (French Edition)

Algorithm for Analysis of Experimental Data



N.B. Statistical Power is: **The Probability of Rejecting a False Statistical Null Hypothesis**